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THE SETTING OF PLASTER OF PARIS

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The setting of plaster of Paris involves a definite chemical transformation from the hemihydrate to the dihydrate of calcium sulfate. Since the former is 4.5 times as soluble as the latter¹ at room temperature, Le Chatelier² assumed the following mechanism for the setting process: The hemihydrate first forms a saturated solution in water, then reacts to form the dihydrate giving a supersaturated solution of the latter from which is deposited a compact mass of interlacing needle-shaped crystals—the set plaster.³ The amount of water necessary to bring the hemihydrate back to the fully hydrated condition is much less than is necessary to dissolve it since a given amount of solution supersaturated with respect to gypsum, deposits crystals of the latter, thereby releasing the water to dissolve another portion of hemihydrate—the process continuing until the transformation to gypsum is complete.

For more than a quarter of a century Le Chatelier's theory of the mechanism of the setting of plaster of Paris was regarded as completely satisfactory. But in more recent years it has been considered by a number of investigators as inadequate to account for all the facts. Following the lead of W. Michaelis⁴ and Keisermann⁵ who observed the formation of a jelly as well as of crystals in the setting of Portland cement, they have visualized the formation of some kind of a jelly as an intermediate stage in the setting of plaster of Paris. Thus, Cavazzi⁶ observed that gypsum precipitated rapidly from aqueous solution with alcohol gave a gelatinous mass from which distinct crystals separated on standing. Without further evidence, he concluded that there was probably an intermediate gel stage in the setting of plaster.

Traube⁷ was the next to suggest that colloidal behavior plays a role in the setting process. It is a well-known fact that soluble salts may have a marked effect on the setting rate,⁸ some salts accelerating it and others retarding it. To account for this behavior Rohland⁹ assumes, in accord with Le Chatelier's theory, that any salt which increases the solubility of calcium sulfate will accelerate the setting while any salt which decreases the solubility will retard the setting. This explanation is inadequate since small amounts

¹ Marignac: *Ann. Chim. Phys.*, (5) 1, 274 (1874).

² "Recherches experimentales sur la constitution des mortieres hydrauliques," Paris (1887); cf., also, van't Hoff et al: *Z. physik. Chem.*, 45, 257 (1903); Rohland: *Z. anorg. Chem.*, 31, 437 (1902); 35, 194; 36, 332 (1903); Jolibois and Chassevent: *Compt. rend.*, 177, 113 (1923).

³ Cf., also, Chassevent: *Ann. Chim.*, 6, 244 (1926).

⁴ *Chem.-Ztg.*, 17, 982 (1893); *Kolloid-Z.*, 5, 9 (1909); 7, 320 (1910).

⁵ *Kolloidchem. Beihefte*, 1, 423 (1910).

⁶ *Kolloid-Z.*, 12, 196 (1913); cf. Neuberg and Rewald: 2, 354 (1908).

⁷ *Kolloid-Z.*, 25, 62 (1919).

⁸ Ditte: *Compt. rend.*, 126, 694 (1898).

⁹ *Z. Elektrochemie*, 14, 421 (1908).

of soluble sulfates decrease the solubility of gypsum and yet increase the rate of set. And there are other exceptions.¹ Traube observed the effect of salts on the time required for the plaster to attain a definite state of hardness. He found cations to be especially important, the order of influence being the reverse of that in which they precipitate sols. This led him to the conclusion, which is not obvious, that there must be some kind of colloidal behavior involved in the setting process.

Ostwald and Wolski² likewise concluded from indirect evidence that colloidal processes are probably involved in the setting of plaster of Paris. Experimentally, they followed the rate of change in viscosity of suspensions of plaster, varying the concentration of the suspensions, the degree of dispersion, the temperature and the nature of the medium, *i. e.*, using solutions of various salts as well as pure water. The theoretical deductions from the experimental data were promised in a second paper which was never published. They merely state that the viscosity data indicate a colloid process, "as for example, the separation of perhaps only a thin gel layer as an integral part of the setting process."

The guarded statement of Ostwald and Wolski that "perhaps only a thin gel layer" was formed at some stage of the setting process together with their failure to discuss theoretically their viscosity data suggests that they were probably in doubt as to whether there was any gel formation at all. In marked contrast to this Baykoff,³ Neville⁴ and Budnikoff⁵ come out definitely in support of the formation of a gel as an intermediate stage in the setting process. Baykoff reached this conclusion as a result of a procedure which he claimed would give a gypsum jelly. Five to ten grams of hemihydrate were mixed with 100 cc of water and shaken vigorously. On stopping the shaking after a suitable time, the entire mass was said to set to a "gelatinous mass presenting the appearance of a silica gel." The setting took place in 2 or 3 minutes if a 10 percent solution of potassium or ammonium sulfate was used instead of pure water. This behavior will be considered in the experimental part of this paper.

Since the hydration of plaster of Paris is an exothermic reaction, the rate of the reaction may be followed by measuring the rate of evolution of heat. This has been done by Cloez,⁶ Emley,⁷ Chassevent,⁸ Neville,⁹ Budnikoff,¹⁰ Hansen¹¹ and others. Starting with a high grade of hemihydrate mixed with pure water and determining the rise in temperature with time, an S-shaped

¹ Haddon: *J. Soc. Chem. Ind.*, 165T, (1920); 122T (1921); cf. Welch: *J. Am. Ceramic Soc.*, **6**, 1197 (1923).

² *Kolloid-Z.*, **27**, 79 (1920); Neugebauer: **31**, 40 (1922).

³ *Compt. rend.*, **182**, 129 (1926); In article by Budnikoff: *Kolloid-Z.*, **42**, 151 (1927).

⁴ *J. Phys. Chem.*, **30**, 1037 (1926).

⁵ *Kolloid-Z.*, **42**, 151 (1927); **44**, 242 (1928).

⁶ *Bull.*, (3) **29**, 171 (1903).

⁷ *Trans. Am. Ceram. Soc.*, **19**, 573 (1917).

⁸ *Ann. Chim.*, **6**, 264 (1926).

⁹ *J. Phys. Chem.*, **30**, 1037 (1926).

¹⁰ *Kolloid-Z.*, **44**, 242 (1928).

¹¹ *Ind. Eng. Chem.*, **22**, 611 (1930).

curve is obtained. For an interval of several minutes the temperature rises but slightly, after which it goes up relatively rapidly to a maximum and then falls off. Neville observed that a so-called "initial set" results before there is any marked heat evolution. He concluded from this that the setting process takes place in two stages: (1) the formation of a gel or adsorption complex between the plaster and water, a process accompanied by but little heat effect and (2) the exothermic reaction between the plaster and the adsorbed water, forming gypsum. At first he attributed the observed contraction in volume to the initial step and the subsequent expansion to the second step, but later² he concluded that the hydration which causes the initial contraction takes place throughout the whole period but it is masked for a time by the thermal expansion.³ The effect of salts on the rate of setting was attributed to their effect on the adsorption of water to form a gel and subsequently to their catalytic action on the reaction between hemihydrate and water.

Budnikoff carried out thermometric observations on the rate of setting of plaster under varying conditions, apparently quite independent of the work of Neville and reached similar conclusions as to the mechanism of the process. There is a distinct difference in the form of Neville's time-temperature curves and those obtained by Budnikoff since the latter, apparently without knowing it, used a plaster containing a large amount of soluble anhydrite. Accordingly there was a marked rise in temperature of 15° to 20° at the outset as a result of the hydration of the anhydrite to hemihydrate.⁴ Budnikoff goes a step further than Neville and postulates the formation of a gel around the plaster particles which protects them from the action of water thereby producing the induction period which varies in length depending on the nature of the addition agents present. The period of induction is assumed to be broken by crystallization of the enclosing gel which allows the water to again act on the plaster. This theory deserves little consideration, for if the facts are as postulated the disappearance of the first gel layer would merely be followed by the formation of a new one giving a second induction period, and so on, the process being repeated indefinitely.

The arguments for gel formation as a step in the setting of plaster of Paris may seem quite conclusive if taken collectively. Indeed one of us⁵ but a short time ago was distinctly impressed by the conclusions of Neville. On reflection it appears, however, that all the evidence of true gel formation is indirect. No one, not even Baykoff, as we shall see, has really observed the formation of a gel of gypsum prior to the appearance of the interlacing crystals in the plaster pastes. Chassevent⁶ independently of Neville or Budnikoff observed an initial inhibition period in the time-heat curves for the hydration of hemihydrate. It probably never occurred to him to invoke the formation

¹ Cf., however, Emley: *Trans. Am. Ceramic Soc.*, **19**, 573 (1917).

² *Colloid Symposium Monograph*, **6**, 309 (1928).

³ Cf. Williams and Westendick: *J. Am. Ceramic Soc.*, **12**, 381 (1929).

⁴ Cloez: *Bull.*, (3) **29**, 171 (1903); Chassevent: *Ann. Chim.*, **6**, 265 (1926)

⁵ Weiser: "The Colloidal Salts," 199 (1928).

⁶ *Ann. Chim.*, **6**, 264 (1926).

of a gelatinous adsorption complex to account for this period of inhibition since he had previously observed an inhibition period in the crystallization of gypsum from its supersaturated solution in the absence of nuclei. Indeed he found that solutions of gypsum containing 5 times the saturation value did not start to crystallize for 28 minutes when particular care was taken to exclude nuclei. The effect of salts on the rate of setting was also observed. In the case of potassium sulfate he states that this "accelerates the crystallization and diminishes the time interval during which the instable saturated solutions of hemihydrate remains without crystallization."

Hansen¹ likewise failed to find any direct evidence of gel formation and apparently independent of Chassevent, reached the same conclusion as the latter that "the effect of foreign material upon the rate of precipitation from its supersaturated solution appears to explain the ability of foreign materials to accelerate or retard the setting of calcined gypsum pastes."

While one can offer no objections to the statements of either Chassevent or Hansen, their conclusion in the last analysis is merely that foreign substances influence the rate of set by influencing the rate at which gypsum precipitates from its supersaturated solution. On the other hand, they offer no explanation of the variation in behavior with various substances. Chassevent does say that substances which increase the sulfate ion concentration accelerate the crystallization; but as we shall see this is not necessarily true.

This communication deals with thermometric and optical observations of the hydration of plaster of Paris under widely varying conditions with the end in view (1) of throwing light on the existence or non-existence of gel formation as a stage in the setting process and (2) of formulating a general theory to account for the effect of addition agents on the rate of setting.

The Question of Gel-Formation in the Setting of Plaster of Paris

1. *Baykoff's gypsum "gel."* As pointed out in the previous section, Baykoff claims to get gypsum as a "gelatinous mass presenting the appearance of a silica gel" by precipitation of the gypsum from a supersaturated solution in water or ammonium sulfate solution. His procedure using an ammonium sulfate solution was repeated: Ten grams of ammonium sulfate were dissolved in 100 cc of water in a 250 cc stoppered bottle and to this was added 10 grams of plaster of Paris. After shaking at intervals, rapidly at first and then more gently for approximately 2 minutes and allowing to stand quietly, it was noted that the mixture gradually became somewhat rigid so that the bottle could be turned upside down without the mass flowing. This was obviously the gypsum "gel" to which Baykoff referred. It possessed but little rigidity and broke completely on gentle stirring and did not reset on standing. Microscopic examination showed it to be a network of relatively long crystal needles of gypsum. The experiment was repeated withdrawing samples of the mixture for microscopic examination at intervals of 30 seconds. After 1.5 to 2 minutes the appearance of myriads of small needles was a reminder that the mixture

¹ Ind. Eng. Chem., 22, 611 (1930).

should be allowed to stand quietly if a "gel" was desired. If the shaking was continued the mass did not "set." The only point of resemblance between this cloudy, non-uniform entangling mass of crystal needles and silica gel is that both stay in the containing vessel when the latter is inverted. Silica gel or jelly, like all true jellies, consists of myriads of ultramicroscopic particles that have adsorbed the solvent strongly and have become enmeshed into a network that entrains liquid.¹ A mass of relatively coarse crystal needles that entangles water constitutes what Holmes² has called a "false gel" in contradistinction to the true gels where the structure is ultramicroscopic. Thus if one dissolves 4-5 grams of caffeine in 100 cc of boiling water and allows the solution to cool slowly, the beaker may be inverted without loss of water. In this case, as in the case of the gypsum, the structure consists of comparatively coarse needle crystals. Everybody is agreed that in the process of setting, plaster of Paris gives an enmeshing network of gypsum needles that entrains the excess water. If this is what people mean when they say that gelation is a step in the setting of plaster of Paris, then one will not question the statement. But this is not what they mean. Neville assumes specifically that the gel is an adsorption complex between the water and the plaster which forms without any chemical change. Budnikoff has the same idea, for he speaks of the initial formation around the plaster particles of a gel layer which subsequently crystallizes.

2. *The Effect of Gypsum Nuclei on the Rate of Set of Plaster of Paris.* It is a well-known fact that samples of high-grade plaster of Paris free from any added accelerators or retarders show considerable variation in the time of set. In general a plaster which exhibits a long period of inhibition before there is any marked rise in temperature, is designated as a slow-setting plaster while one with a short period of inhibition is referred to as a rapid-setting plaster. If the period of inhibition is due to the building up of an adsorption complex "whereby the two reactants are brought into chemical contact," as Neville assumes, then it is not obvious why different samples prepared by similar procedures and having the same average particle size, should show such differences in the period of inhibition. On the other hand, if the inhibition period is merely a phenomenon of supersaturation, the variation in the length of the period with different samples of plaster might well be due to variation in the number of gypsum nuclei in the samples. Chassevent³ showed that the addition of gypsum to plaster hastened the time of set and Hansen⁴ found that if a plaster paste was made with water shaken for 35 minutes with a small amount of plaster which ordinarily attained its maximum temperature in 75 minutes, the time of setting was appreciably shortened.⁵ But the importance of the presence of gypsum nuclei in plaster of Paris on the rate at which it sets has been pretty generally overlooked especially by everybody who has visualized gel formation as a stage in the setting process.

¹ Weiser: Bogue's "Colloidal Behavior," 1, 393 (1924).

² Colloid Symposium Monograph, 1, 24 (1923).

³ Ann. Chim., 6, 313 (1926).

⁴ Ind. Eng. Chem., 22, 611 (1930).

⁵ Cf. Wiggins Sons Co., British Pat., 221,853 (1923).

In this as in succeeding experiments on the rate of set of plaster, the thermometric method was employed. A diagram of the apparatus used is shown in Fig. 1. This consists of a Dewar vacuum vessel 8.2 cm internal diameter and 29 cm deep. The vessel is supplied with a snug-fitting cork stopper attached to a board which rests on the top of the vessel when the

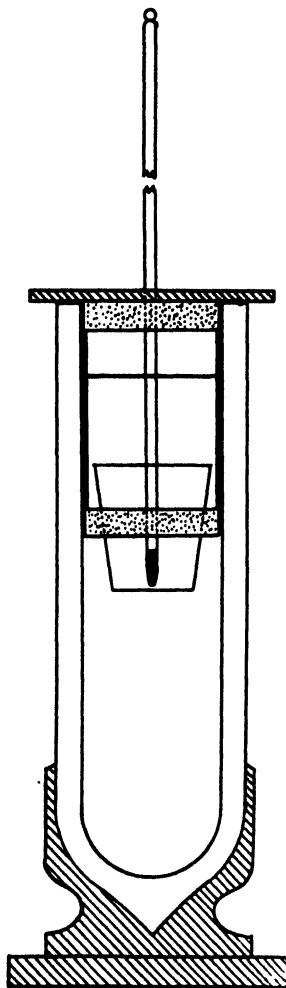


FIG. 1
Diagram of Calorimeter.

stopper is inserted. To the stopper is suspended firmly a cork ring which holds the paraffined paper cup containing the plaster paste. A hole through the center of the stopper admits a 100° thermometer graduated in tenths of a degree.

The procedure using this apparatus is as follows: Into a 130 cc paraffined paper cup is measured exactly 35 cc of distilled water kept in the thermostat at 25°. A 50-gram sample of the plaster likewise kept at 25° was put into the cup and the stop-watch started. By brisk stirring for 10 seconds with a glass rod, a uniform paste was obtained. The cup with contents was put in the holder, the thermometer inserted in the paste which was then covered with a thin layer of paraffine oil to prevent evaporation. After placing in the Dewar vessel, the temperature was read at 1 minute intervals until after the maximum temperature was attained and the temperature began to fall regularly.

The plaster of Paris used in these experiments was a high grade product secured from the Central Scientific Company. The rate of set of the plaster was somewhat too rapid but it was found that this was decreased by ignition. Accordingly 2-kilogram samples in a flat tin container were placed in an electric oven at 130° for two hours, stirring thoroughly every 30 minutes. Approximately 40 kilograms were treated in this way and the entire amount was spread out thinly on paper in a closed room in which the humidity was kept high. This served to transform into hemihydrate any anhydrite that was formed during the ignition. After thorough mixing, following the standard procedure,¹ the plaster was transferred to tightly corked bottles. Unless otherwise stated this "Standard Sample" was used in all experiments described herein.

The time-temperature curves were run on (1) a sample of the original plaster that had been heated at 130° for 5 hours and allowed to stand in the air over night, (2) on the standard sample described above, and (3) on the

¹ Treadwell-Hall: "Analytical Chemistry," 2, 53 (1924).

standard sample to which varying amounts of gypsum were added. The gypsum used was set plaster which was finely ground to pass a 100 mesh sieve. The weighed sample was thoroughly mixed with the 50 gram weight of plaster before the water was added. The time-temperature curves for various mixtures given in Table I are shown in Fig. 2.

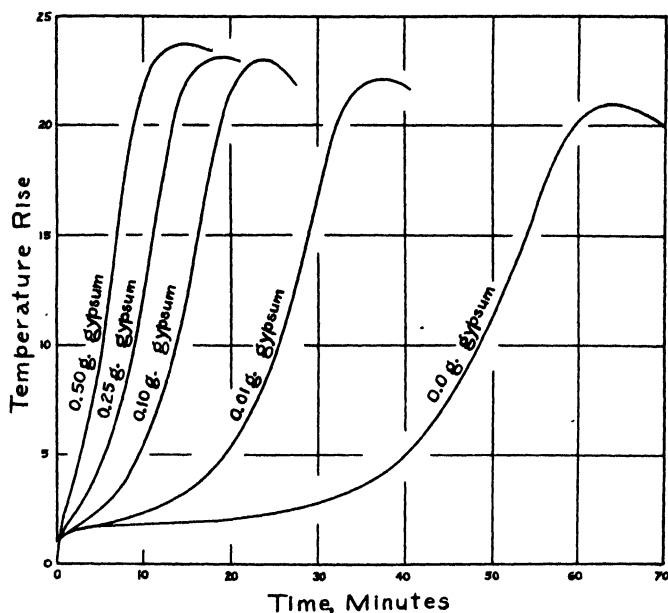


FIG. 2

Time-Temperature Curves obtained with Different Samples of Plaster of Paris.

TABLE I

Observations on Different Samples of Plaster of Paris

Substances mixed with 35 cc water Plaster of Paris 50 grams	Grams gypsum		Time to attain maximum temperature	Tensile strength pounds per square inch	
	added	calculated		after 1 day	after 10 days
Sample heated 45 hours	0.0	0.00012	100.0		
Standard sample	0.0	0.00092	64.0	235	490
Standard sample	0.01	0.0098	38.0		
Standard sample	0.05	0.052	26.5		
Standard sample	0.10	0.08	24.0		
Standard sample	0.25	0.23	19.0		
Standard sample	0.50	0.50	16.0	300	450
Standard sample	1.00	0.94	14.0		

From the form of the curve with a plaster to which no gypsum was added it will be noted that there is a sharp rise of approximately 1° which is probably due to heat of wetting and to the transformation of any anhydrite into hemihydrate. The initial rise is followed by a "period of inhibition" after which

there is a gradual increase in rate of reaction until a maximum temperature is attained. The sample heated for 5 hours (curve not shown) which was quite free from nuclei did not attain the maximum temperature for 100 minutes while the standard sample showed a shorter inhibition period and reached the maximum temperature in 65 minutes. The inhibition period was appreciably cut down by adding nuclei of gypsum until with 0.5 gram in 50 grams of plaster, it was practically zero. When the time to attain the maximum temperature is plotted against the weight of gypsum nuclei added a parabolic curve is obtained, Curve I, Fig. 3, which shows that the rate of set approaches

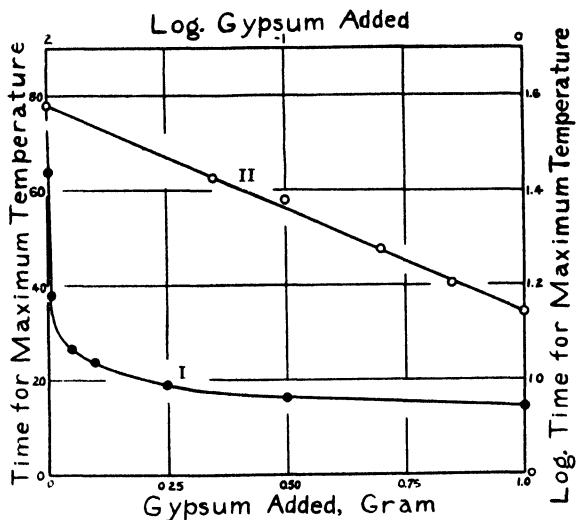


FIG. 3

Effect of the addition of Gypsum Nuclei on the Rate of Set of Plaster of Paris.

infinity as the number of nuclei present approaches zero. Plotting the data on logarithmic coordinates the straight line, Curve II, of Fig. 3, is obtained. The equation for the curve is $W = 1.52 \times 10^{5t-4.55}$, where W = grams gypsum added and t = time to attain maximum temperature. From this the values for the weight of nuclei added may be calculated. For the purposes of comparison with the actual amounts added, the calculated values, which show close agreement, are given in the third column of Table I. Using the equation, the amount of nuclei present in the two samples to which no gypsum was added can be calculated. It is interesting to note that the calculated value for the very slow setting plaster is approximately 0.0001 gram per 50 grams while for the standard sample it is less than 0.001 gram per 50 grams.

The above observations indicate that the length of the inhibition period is influenced to a marked degree by the amount of gypsum nuclei in the plaster paste after the mixing with water is complete. Hence the observed inhibition period appears to result from delayed precipitation from a supersaturated solution owing to dearth of nuclei rather than to the time necessary to form a gel or adsorption complex.

It cannot be too strongly emphasized that the period of inhibition even in a relatively slow setting plaster, is not a period of rest so far as chemical change is concerned. The temperature rises gradually throughout the entire period, showing the precipitation of more and more gypsum on nuclei already present and the formation of new nuclei until there is rapid precipitation of gypsum followed by further solution and hydration of hemihydrate throughout the mass. This was confirmed by a motion picture record of the process magnified 30 times. The absence of temperature rise, or an actual drop in temperature, during the first stages of the process, is due either to loss of heat by evaporation of water from the paste or radiation owing to insufficient insulation in a room where the temperature is lower than that of the newly prepared paste.

In view of the fact that there is a gradual increase in the viscosity of the plaster paste from the time of its formation, it is not possible to indicate any one point where the plaster begins to harden and to designate it as the "initial set." Emley¹ states specifically that none of the methods such as measurements of expansion or of temperature rise, is capable of indicating an "initial set."

In the last two columns of Table I are given the tensile strengths,² after 1 day and after 10 days, of the standard plaster with and without the addition of a small amount of gypsum. It will be noted that the unseeded sample which sets slowly and the seeded sample which sets rapidly, attain approximately the same strength after 10 days; but the rapid-setting sample has a higher strength after 1 day than the slow-setting sample. It would seem from these observations that suitable ignition to eliminate gypsum nuclei is all that is necessary to obtain slow-setting plaster of Paris and that the rate of set can be increased to any desired point by seeding with a suitable amount of finely powdered gypsum.

3. *Effect of stirring Plaster-Water Mixtures on the Rate of Set.* It is a well-known fact that stirring a mixture which has the property of setting to a uniform jelly structure will prevent or at least greatly retard the gel formation. If the setting of plaster of Paris involves the initial formation of a jelly, it would follow that the process and the subsequent set would be retarded by agitation of the plaster-water mixture. On the other hand, if the phenomenon consists merely of dissolution and hydration of hemihydrate followed by precipitation of gypsum from its supersaturated solution, and the usual period of inhibition is due to a scarcity of gypsum nuclei, it would follow that stirring would tend to break down the supersaturated solution, supplying nuclei which would decrease the length of the induction period and hence the time of set. As a matter of fact, the latter is what happens as the following experiments show. Fifty-gram samples of the standard plaster were mixed rapidly with 35 cc of water in a paraffined paper cup. The pastes were subjected to rapid agitation for varying periods of time, using a Central Scientific Company motor-driven stirrer No. 12860 running at maximum speed. The stirrer was a metal disc of 6 paddles, 3 cm. in diameter. The time-temperature curves

¹ Trans. Am. Ceramic Soc., 19, 573 (1917).

² Determined by the standard procedure of the American Society for Testing Materials.

obtained in the usual way for the several samples are reproduced in Fig. 4. Note especially the shortening of the inhibition period with increasing time of stirring. A lower maximum temperature was recorded for the mixtures stirred 3 and 4 minutes than for those stirred a shorter time because of loss of heat before placing the mixture in the calorimeter. The results are summarized in Table II and shown graphically in Fig. 5. It is significant that the form of the curve is similar to that obtained by the direct addition of varying amounts of gypsum to the plaster.

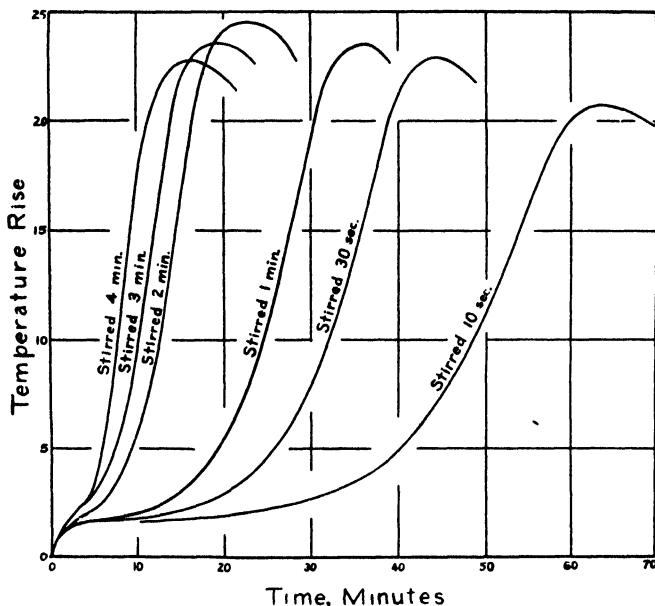


FIG. 4

Time-Temperature Curves for Plaster of Paris Pastes Stirred for Varying Periods.

TABLE II

Effect of Stirring on the Rate of Set of Plaster of Paris

Time of stirring with motor driven stirrer	Time for maximum temperature
10 seconds (by hand)	64
30 seconds	44
1 minute	36
2 minutes	22
3 minutes	19
4 minutes	17
5 minutes	Set before the thermometer was introduced

These three sets of observations individually and collectively furnish strong evidence in support of the view that the inhibition period is a phenomenon of supersaturation rather than of gel formation.

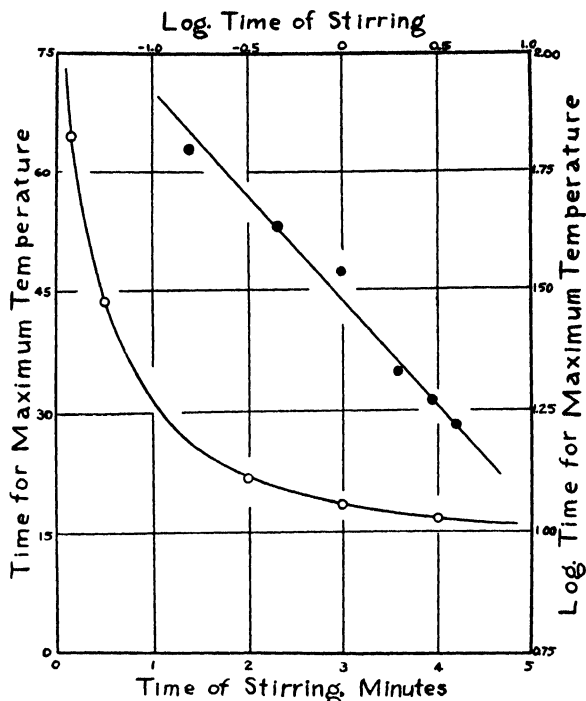


FIG. 5

Effect of Time of Stirring on the Rate of Set of Plaster of Paris.

Effect of Foreign Electrolytes on the Rate of Setting of Plaster of Paris

Since the setting of plaster of Paris involves the precipitation of gypsum from its supersaturated solution, the effect of electrolytes on the process may be considered in the light of von Weimarn's theory of the precipitation process.¹ Von Weimarn calls attention to the fact that there are a number of factors on which precipitation depends, the most important of which are (1) the solubility of the precipitating substance and (2) the concentration at which the precipitation begins. The process of precipitation is considered as taking place in two stages: the first stage in which the molecules condense to invisible or ultra-microscopic particles; and the second, which is concerned with the growth of the particles as a result of diffusion.

Considering the first stage, the velocity W at the first moment can be formulated

$$W = K \frac{\text{precipitation pressure}}{\text{precipitation resistance}} = K \frac{Q - L}{L} = K \frac{P}{L} = KU$$

¹ "Zur Lehre von den Zuständen der Materie" (1914).

in which K is a constant, Q the total concentration of the substance that is to precipitate and L the ordinary solubility of the substance. $Q - L = P$, the amount of supersaturation and $P/L = U$, the percentage supersaturation. A given value of U will result either from a large P value or a small L value. In the first case a large amount of precipitate will be thrown down in a given time while in the second a relatively small amount will form.

The velocity V of the second stage of the process is given by the Nernst-Noyes equation

$$V = D/S \cdot O \cdot (Q - L)$$

where D is the diffusion coefficient, S the length of the diffusion path, O the surface, Q the concentration of the surrounding solution and L the solubility of the dispersed phase for a given degree of dispersity. As in the von Weimarn equation $Q - L$ is the absolute supersaturation.

This general statment of the conditions which obtain during precipitation from solution may now be applied to the precipitation of gypsum from its solution both in the presence and in the absence of foreign electrolytes. The solubility of plaster of Paris in water is approximately 0.067 mol per liter¹ and when this hydrates to gypsum which has a solubility of but 0.015 mol per liter, the percentage supersaturation is $\frac{0.067 - 0.015}{0.015} = 3.5 = U$. The initial velocity of precipitation is proportional to U , that is, $W = K 3.5$. In view of the relatively long period of inhibition following the mixing of pure plaster of Paris with water, it is obvious that this percentage supersaturation is insufficient to cause rapid precipitation of nuclei which must be present in abundance for a rapid reaction to take place throughout the mass. Now if the addition of a foreign electrolyte cuts down the period of inhibition it follows that the percentage supersaturation of the solution with respect to gypsum must be greatly increased. This may be accomplished in one of two ways: Either the solubility of the hemihydrate is increased appreciably more than that of gypsum by the presence of the foreign electrolyte or the solubility of the gypsum is decreased appreciably more than that of hemihydrate by the presence of the foreign electrolyte. In the first instance the value of $(Q-L)/L = P/L$, the percentage supersaturation, is increased because the value of Q , which is determined by the solubility of hemihydrate is increased proportionately more than L the solubility of the gypsum in the medium; and in the second case P/L is increased because L is decreased proportionately more than P in the given medium. In other words for a foreign electrolyte to change the initial rate of formation of nuclei as compared to the rate of formation in water alone, all that is necessary, other things being equal, is for the ratio of the solubility of hemihydrate to the solubility of gypsum to be greater or less than 4.5, the ratio of the solubilities in pure water. There is of course no reason to expect a constant ratio of solubilities in widely different environments but direct evidence of such variation in the solubility ratios are quite impossible to get in most cases since solutions of foreign electrolytes added to hemi-

¹ Marignac: Ann. Chim. Phys., (5) 1, 274 (1874).

hydrate usually result in such rapid precipitation of gypsum that the solubility of hemihydrate in the solution cannot be determined accurately. On the other hand, the observations recorded in the subsequent paragraphs furnish strong indirect evidence of the expected variation in the solubility ratio and in one case this has been evaluated experimentally.

Coming back to the question of growth of particles on nuclei already present, the Nernst-Noyes formulation states that the velocity of growth is

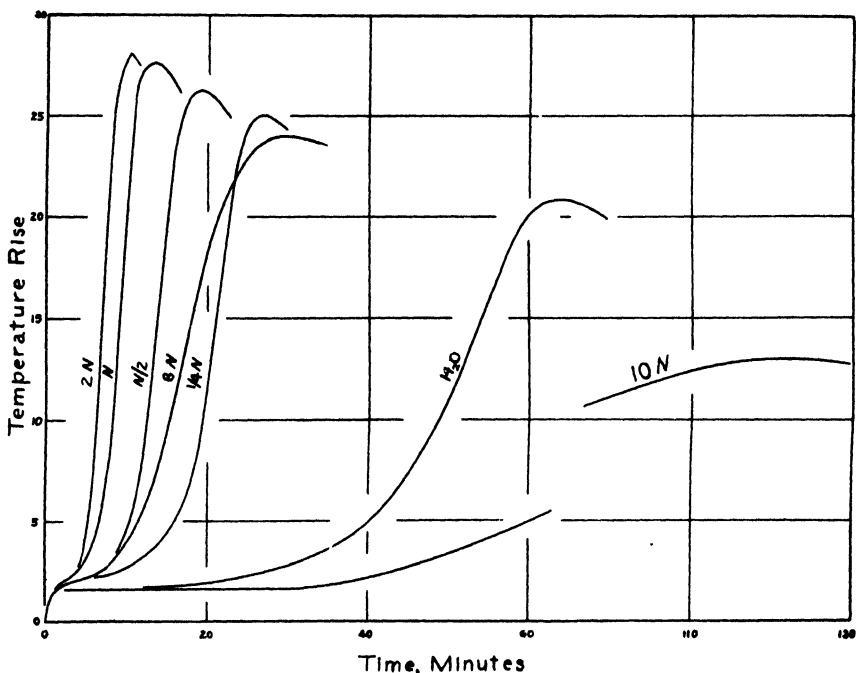


FIG. 6

Time-Temperature Curves for Mixtures of Plaster of Paris with Varying Concentrations of NH_4NO_3 .

proportional to $Q - L$, the absolute supersaturation. For a given amount Q in solution, the velocity of growth is influenced strongly by the solubility. If L is small, so that the $Q - L$ value is relatively large, the growth of particles will be relatively rapid; while if L is large so that $Q - L$ is relatively small, well formed crystals will form slowly. As is well known, for the growth of large well-formed crystals, the $Q - L$ value must be very small and there must be few nuclei on which precipitation takes place.

In the subsequent experiments the effect on the rate of set of plaster of Paris is determined for a few typical salt solutions and is considered in the light of the theory above outlined.

Effect of Ammonium Nitrate. Since ammonium nitrate is quite soluble and the solubility of gypsum in a wide range of concentration is known, the effect of this salt on the rate of set of plaster of Paris was first studied. Fifty-

gram portions of the plaster were mixed for 10 seconds with various concentrations of salt solutions and the time-temperature curves obtained for the several mixtures. To give an idea of the way in which the form of the curve varies with different concentrations of salt, a few of the curves are reproduced in Fig. 6. It is clear from these curves that for concentrations in the neighborhood of 1 to 2 molar the inhibition period is very small and the rate of set is quite rapid. With concentrations below normal the inhibition period and the rate of set become gradually longer approaching that of pure water. The same

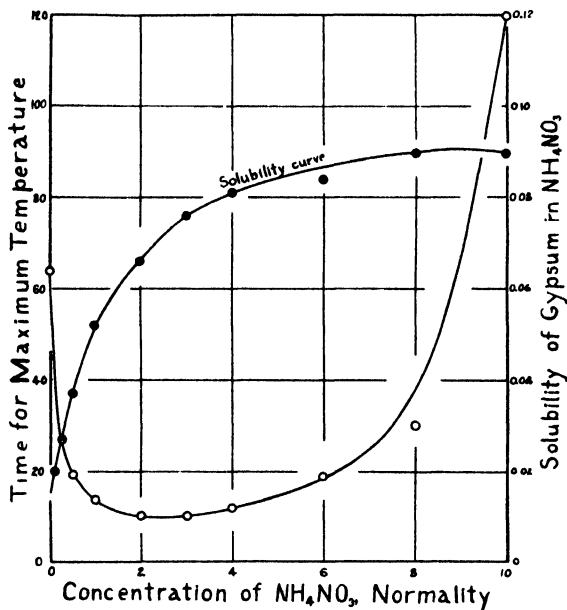


FIG. 7

Effect of the Concentration of NH_4NO_3 Solutions on the Rate of Set of Plaster of Paris.

is true for higher concentrations as evidenced particularly by the behavior in 10 normal solution where the period of inhibition is much longer than in pure water and the time for the maximum temperature to be reached is twice as long as in water. The data are summarized in Table III and shown graphically in Fig. 7. The solubility curve of gypsum in ammonium nitrate solution¹ is given for reference. The U-shaped form of the rate of set curve indicates that with low concentrations of nitrate solution, the ratio of the solubility of hemihydrate to gypsum is sufficiently large that a high percentage supersaturation of gypsum obtains. This results in prompt precipitation of nuclei and because of relatively high absolute supersaturation the growth of the crystals goes on rapidly until the reaction is complete. On the other hand, in strong nitrate solution in which gypsum is quite soluble, $Q - L = P$ is small

¹ Cameron and Brown: J. Phys. Chem., 9, 210 (1905).

TABLE III
Effect of NH_4NO_3 on the Setting of Plaster of Paris

Concentration of NH_4NO_3 solution normality	Time for maximum temperature	Solubility of gypsum from curve of Cameron and Brown ¹ Mol per liter	Tensile strength pounds/sq. in.	
			after 1 day	after 10 days
0.0	64	0.015	235	490
0.10	—	0.020	225	300
0.25	27	0.027		
0.5	19	0.037		
1.0	13.5	0.052	115	200
2.0	10.5	0.066		
3.0	10.0	0.076		
4.0	12.0	0.081		
6.0	19.0	0.084	60	40
8.0	30.0	0.089		
10.0	121.0	0.089	60	40

and P/L is small so that the initial formation of nuclei and the subsequent growth of crystals is greatly retarded.

The correctness of the above interpretation of the effect of varying concentrations of ammonium nitrate on the rate of set of plaster of Paris, is indicated further by optical observations of the form of the gypsum crystals obtained under varying conditions. The procedure was as follows. Exactly 1 milligram of the standard plaster was placed on a microscope slide and to this was added from a 2 cc graduated pipette, 0.1 cc of water or solution. After mixing, the sample was covered with a cover glass, taking care to avoid entrapping air bubbles. To prevent loss of water by evaporation, a film of paraffine was painted around the edge of the cover glass using a small camel's hair brush. After the transformation to gypsum was complete, a microphotograph of the resulting crystal was taken. Six of these photographs are reproduced in Fig. 8. Note that with relatively low concentration of NH_4NO_3 , the needle crystals of gypsum are relatively fine. This means that the percentage supersaturation at the outset is so high that a great number of nuclei are formed and a relatively large number of small needles result. With increasing concentration of nitrate in which gypsum is more soluble, the percentage supersaturation at the beginning of the process falls off and the absolute supersaturation is reduced so that fewer, larger crystals result. This difference in size and shape of the crystals becomes quite marked in 6 N NH_4NO_3 and is particularly striking in 8 and 10 N NH_4NO_3 . The very large crystals obtained in the 10 N NH_4NO_3 solution form quite slowly on few nuclei.

It should be remembered that for a complete quantitative formulation of the rate of formation and the nature of the precipitate as it is affected by the solubility of the substances concerned, it would be necessary to know the

¹J. Phys. Chem., 9, 210 (1905).

solubility of plaster of Paris in varying concentrations of ammonium nitrate throughout the range. It is obvious that such data cannot be secured with low concentrations of NH_4NO_3 because of the rapid rate of transformation of the hemihydrate to gypsum in the nitrate solution. On the other hand with 10 N NH_4NO_3 solution, the rate of transformation is relatively slow, so that it

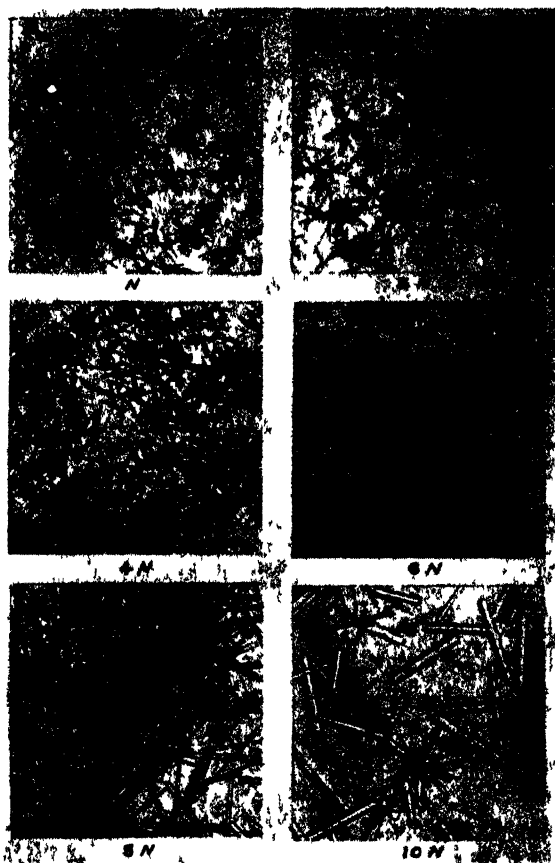


FIG 8
Photomicrographs of Gypsum Crystals obtained in Various
Concentrations of NH_4NO_3 ($\times 40$)

is possible to make a fairly accurate determination of the solubility of plaster of Paris in this solution. The procedure was as follows: approximately 10 grams of plaster was shaken for 5 minutes with 100 cc of 10 N NH_4NO_3 . After centrifuging for a minute to throw down the excess plaster the solution was filtered rapidly through a Gooch crucible and a 50 cc portion of the filtrate heated to boiling and precipitated with BaCl_2 solution. After digesting hot over night, the precipitated BaSO_4 was collected in a Gooch crucible, ignited and weighed. On account of the contamination of BaSO_4 by adsorbed nitrate, the precipitate obtained was probably slightly heavier than it should have

been. However, the same procedure was used by Cameron and Brown¹ to determine the solubility of gypsum in nitrate solutions, so that the two determinations are comparable.

The solubility of plaster of Paris in 10 *N* NH_4NO_3 was found to be 15.5 grams CaSO_4 per liter or 0.114 mol per liter as compared with 0.089 mol per liter for the solubility of gypsum in the salt solution. The ratio of solubilities is thus 1.3 as compared with 4.5 in pure water and the $(Q-L)/L = P/L$ value in the nitrate solution is 0.3 as compared with 3.5 in pure water. These data furnish a quantitative basis for the above explanation of the observed differences in behavior of plaster of Paris in different solutions.

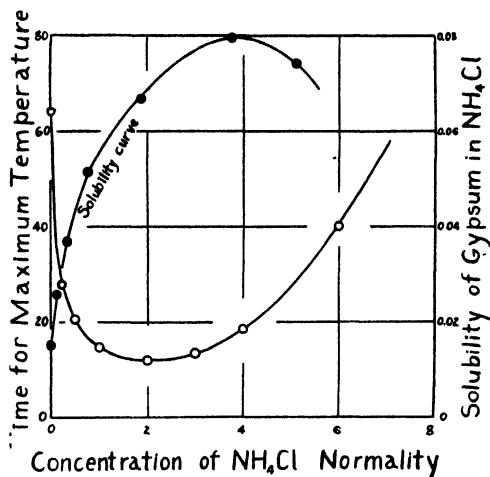


FIG. 9
Effect of the Concentration of NH_4Cl Solutions on the Rate of Set of Plaster of Paris.

TABLE IV

Effect of NH_4Cl on the Setting of Plaster of Paris

Concentration of NH_4Cl solution normality	Time for maximum temperature	Solubility of gypsum from the curve of Cameron and Brown ¹ Mols per liter
0.0	64	0.015
0.25	28	0.031
0.50	20.5	0.043
1.00	14.5	0.055
2.0	12.0	0.069
3.0	13.0	0.077
4.0	18.5	0.079
6.0	40.0	0.071

¹ J. Phys. Chem., 9, 210 (1905).

Referring to the last two columns of Table III, it will be seen that the tensile strength of the set plaster formed in the presence of NH_4NO_3 solutions is less than that formed in pure water. This is especially true when the nitrate solution is so strong that relatively few crystals of gypsum result.

Effect of Ammonium Chloride. The behavior of plaster of Paris in ammonium chloride solutions is essentially the same as with ammonium nitrate, as indicated by the results of the observations recorded in Table IV and shown graphically in Fig. 9. The curve has the same form as that obtained with nitrate but it runs slightly higher indicating that the initial percentage supersaturation with respect to gypsum is somewhat lower in the ammonium chloride than in the corresponding nitrate solutions. This was confirmed by a comparison of the microphotographs of the crystals obtained with like concentrations of the electrolytes.

Effect of Ammonium Sulfate. The effect of varying concentrations of ammonium sulfate on the rate of transformation of hemihydrate to gypsum is shown by the results tabulated in Table V which have been plotted in Fig. 10. It will be noted that the rate of hydration of the plaster, as evidenced by the time for attaining the maximum temperature, is greater than that with equivalent solutions not only of NH_4Cl and NH_4NO_3 but of all the salts investigated. As has been frequently pointed out, this rapid rate of reaction is contrary to Rohland's theory which attributes the increased rate of set of plaster in salt solutions to the increase in solubility of gypsum in these solutions, since the solubility curve reproduced in Fig. 10 discloses a smaller solubility of gypsum in low concentrations of $(\text{NH}_4)_2\text{SO}_4$ solutions than in water. As a matter of fact, the effect of the salt on the solubility of gypsum is in itself altogether insufficient to explain the effect of the salt on the rate of hydration of plaster. The important thing is the ratio of the solubility of plaster to that

TABLE V
Effect of $(\text{NH}_4)_2\text{SO}_4$ on the Setting of Plaster of Paris

Concentration of $(\text{NH}_4)_2\text{SO}_4$ solution normality	Time for maximum temperature	Solubility of gypsum from curve of Sullivan ¹ Mols per liter	Tensile strength pounds/sq. in.	
			after 1 day	after 10 days
0.0	64.0	0.015	235	490
0.25	18.0	0.011		
0.50	11.0	0.011		
0.75	8.0	0.013		
1.00	7.5	0.014	165	425
1.50	7.0	0.017		
2.0	6.5	0.019		
3.0	7.0	0.025		
4.0	8.0	0.028		
6.0	14.0	0.031	160	475
7.0	16.5	0.032	150	385
8.0	20.0	0.033		

¹ J. Am. Chem. Soc., 27, 529 (1905).

of gypsum in a given concentration of the salt, since this ratio determines the initial percentage supersaturation which influences greatly the rate of formation of nuclei and the absolute supersaturation which determines the rate of growth. The form of the curve in Fig. 10 indicates that the ratio of solubility of plaster to gypsum is high even in relatively low concentrations of $(\text{NH}_4)_2\text{SO}_4$. This would account for the absence of a period of inhibition in the time-temperature curves since $(Q-L)/L$ would be large; and would account also for the rapid rate at which the reaction once started goes to completion since $Q-L$ would be relatively large. That such is the case would follow also from the

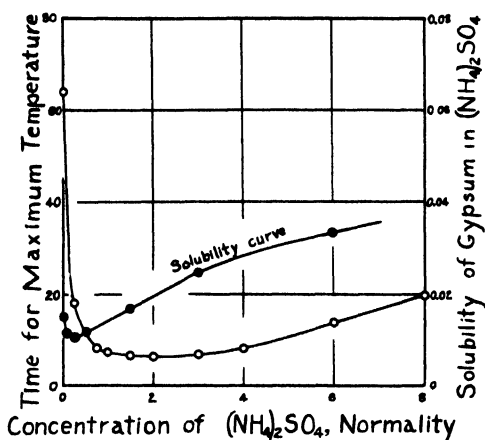


FIG. 10
Effect of the Concentration of $(\text{NH}_4)_2\text{SO}_4$ Solutions
on the Rate of Set of Plaster of Paris.

form of the particles obtained in the solutions of varying strengths, photomicrographs of which are given in Fig. 11. Note the relatively small size of the crystals formed in the zone of very rapid set in contact with solutions between N and $4N$. In $6N$ solutions the crystals which form somewhat slower are larger while in slightly higher concentrations a mat of long needles results. The lower $Q-L = P$ value and P/L value in solutions between 7 and $8N$ in which the solubility of gypsum is twice what it is in water, manifest themselves in a slower time of set and the formation of larger much longer needles.

The tensile strength data in Table V show that ageing the plaster which sets rapidly in the presence of sulfate gives a product which approaches in strength the product formed with pure water.

Effect of Ammonium Thiocyanate. Observations on the effect of NH_4CNS on the rate of formation of gypsum from plaster are summarized in Table VI. The curve representing the effect of the thiocyanate concentration on the time for attaining the maximum temperature is given in Fig. 12 together with similar curves for the other salts investigated. The photomicrographs of the gypsum crystals formed in the presence of varying concentrations of the salt

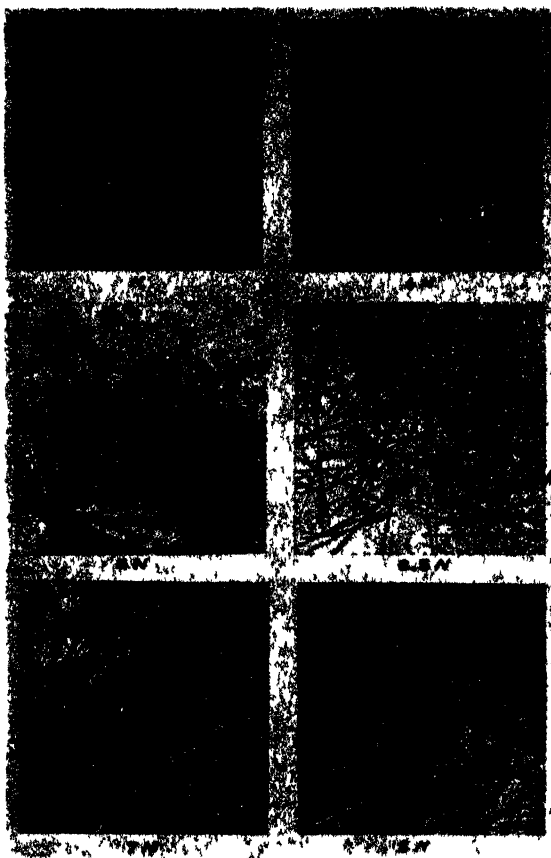


FIG. 11
Photomicrographs of Gypsum Crystals obtained in Various Concentrations of $(\text{NH}_4)_2\text{SO}_4$ ($\times 40$)

TABLE VI

Effect of NH_4CNS on the Setting of Plaster of Paris

Concentration of NH_4CNS solution normality	Time for maximum temperature	Concentration of NH_4CNS solution normality	Time for maximum temperature
0.50	23.0	4.0	26.25
1.0	17.5	5.0	46.0
2.0	15.75	6.0	80.0
3.0	18.50		

are reproduced in Fig. 13. These data merely emphasize the importance of the degree of supersaturation on the rate of formation and the form of the crystals.

It is of interest to compare the crystal mass obtained in the presence of 6.5 *N* $(\text{NH}_4)_2\text{SO}_4$ with that formed in the presence of 6 *N* NH_4CNS . Although, at first glance they may appear quite similar, there is actually a distinct difference in the appearance as a result of a difference in the conditions leading to their formation. With the thiocyanate solution there was a marked period of inhibition in the time-temperature curve while with the sulfate solution there was little or no inhibition period. This indicates that

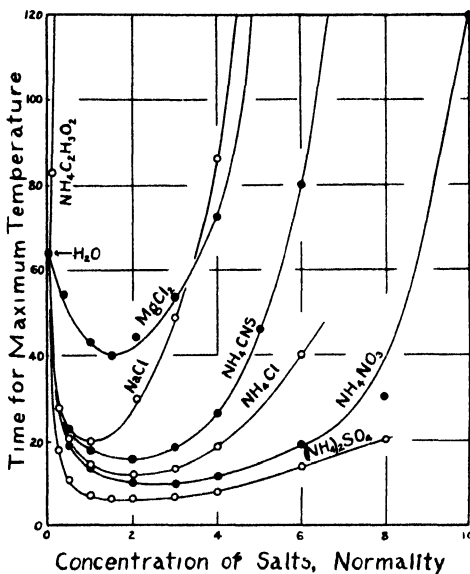


FIG. 12
Effect of Varying Concentrations of Several Electrolytes
on the Rate of Set of Plaster of Paris.

the percentage supersaturation is greater in the latter case. The actual supersaturation was also greater with $(\text{NH}_4)_2\text{SO}_4$ than with NH_4CNS since a very large number of fine needles formed rapidly with the former and a smaller number of thicker needles formed more slowly with the latter.

TABLE VII

Effect of NaCl on the Setting of Plaster of Paris

Concentration of NaCl solution normality	Time for maximum temperature	Solubility of gypsum from curves of Cameron. ¹ Mols per liter
0.0	64	0.015
1.0	20	0.046
2.0	30	0.054
3.0	49.5	0.055
4.0	86	0.052
Saturated	251	0.040

¹ J. Phys. Chem., 5, 556 (1901).

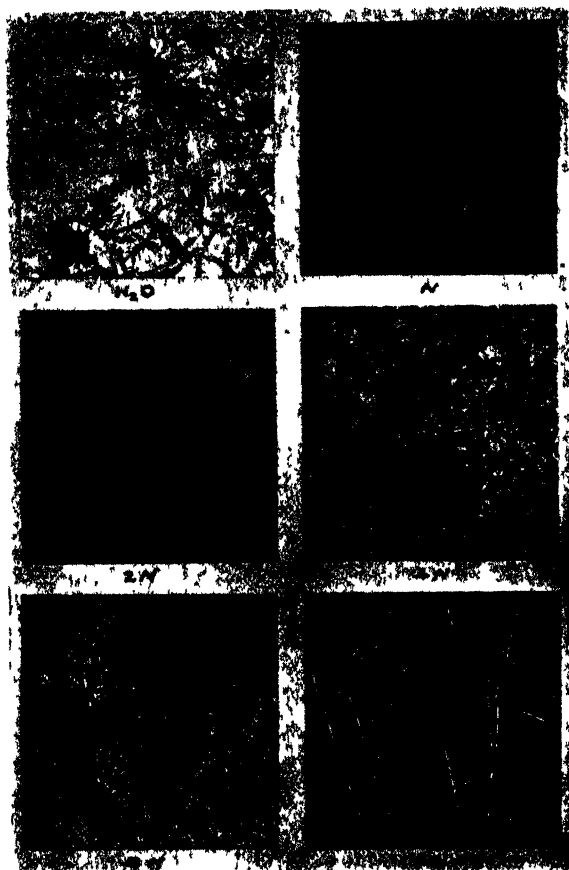


FIG. 13
Photomicrographs of Gypsum Crystals obtained in Various
Concentrations of NH_4CNS ($\times 40$)

TABLE VIII

Effect of MgCl_2 on the Setting of Plaster of Paris

Concentration of MgCl_2 solution normality	Time for maximum temperature	Solubility of gypsum from curve of Cameron and Seidell ¹ Mols per liter
0 0	64 0	0 015
1 0	43 5	0 056
1 5	40 0	0 061
2 0	45 0	0 063
3 0	53 5	0 063
4 0	72 0	0 054
5 0	180 0	0 035

¹ J. Phys Chem, 5, 643 (1901).

Effect of Sodium Chloride. The effect of sodium chloride on the rate of set of plaster is similar in all essential respects to that of the ammonium salts as indicated by the data recorded in Table VII and plotted in Fig. 12.

Effect of Magnesium Chloride. Observations of the time required to reach the maximum temperature when varying concentrations of magnesium chloride solution are mixed with plaster of Paris are given in Table VIII and plotted with the other curves in Fig. 12. It is obvious that the general form of the curve is the same irrespective of whether a salt of a divalent or of a univalent cation is employed.

Effect of Ammonium Acetate. Certain organic salts such as ammonium acetate and ammonium citrate have been called retarders since they were found to slow down the rate of set of plaster of Paris even when present in low concentrations. This observation was confirmed with ammonium acetate as shown by the data recorded in Table IX from which the curve in Fig. 12 was obtained.

TABLE IX
Effect of $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ on the Setting of Plaster of Paris

Concentration of $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ solution normality	Time for maximum temperature	Solubility of gypsum estimated from data by Cohn ¹ Mol per liter	Tensile strength pounds/sq.in.	
			after 1 day	after 10 days
0 0	64	—	235	490
0 04	64	—	—	—
0 10	83	0.06	100	240
0 50	240	0.15	125	250

The reason for the marked retarding action of $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ solution in concentrations as low as $N/10$ and for the extremely low rate of reaction in $N/2$ solution calls for special consideration.

On several occasions² attention has been called to the fact that the rate of formation of a precipitate and the nature of the precipitate is not regulated generally and uniformly by its solubility and the supersaturation prevailing in accord with the theory of von Weimarn. The latter has taken care of variations from his theory by the introduction into his equations of what he calls "variable multipliers" the value for any substance being "the product of all other factors (in addition to P/L) which influence the crystallization process." This statement of von Weimarn is a frank admission that factors other than solubility do come in; but his formulation is of no help in solving the problem as to what factor or factors other than the solubility of hemihydrate and gypsum in ammonium acetate accounts for the apparently abnormal behavior of the plaster in contact even with low concentrations of the solution.

¹ J. prakt. Chem., (2) 35, 43 (1887).

² Weiser and Bloxson: J. Phys. Chem., 28, 26 (1924); Weiser and Cunningham: 33, 301 (1929); Weiser: "The Colloidal Salts", 1 (1928).

Photomicrographs of the crystals of gypsum obtained in dilute ammonium acetate solutions are reproduced in Fig. 14. It will be noted that the crystals are quite different in appearance from the needle-like crystals usually obtained. Those grown in the $N/10$ solution are short thick crystals while those grown in the $N/2$ solution consist of very thin hexagonal plates. This difference in appearance suggested the possibility that the abnormal behavior in acetate solutions might be due to a difference in the crystal structure of the gypsum formed.

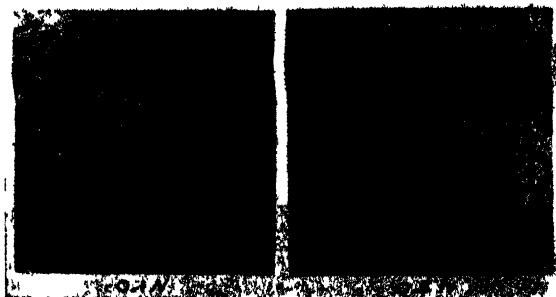


FIG. 14
Photomicrographs of Gypsum Crystals obtained in 0.1 Normal and 0.5 Normal Ammonium Acetate ($\times 40$).

X-ray Analysis of Gypsum

To determine whether the gypsum formed in acetate solution possessed the same structure as that obtained in pure water the x-ray diffraction patterns of different samples were made with the General Electric X-ray Diffraction Apparatus. At the same time the crystals from other solutions were subjected to x-ray analysis to determine whether or not they were chiefly gypsum. Thus the crystals formed in strong solutions of NH_4NO_3 and $(\text{NH}_4)_2\text{SO}_4$ were so different in appearance from the usual small needle crystals that it was not certain but that the precipitates were double or complex salts. The crystals for analysis were prepared by mixing plaster and solutions in the same ratio as was used in preparing the slides for optical observations. The mixture was spread out thinly in a crystallizing dish and placed in a covered vessel containing water to prevent evaporation. After the reaction was complete (in the case of the $N/2$ $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$, after 2 days) the crystals were washed, dried, placed in a small capillary tube and the x-radiograms prepared by 8 hours' exposure. In Fig. 15 are reproduced photographs of the diffraction patterns for (1) plaster of Paris; and of the products formed by the action of plaster with (2) water, after 1 month (3) water, after 1 hour (4) 0.5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ (5) 10 N NH_4NO_3 (6) 7 N $(\text{NH}_4)_2\text{SO}_4$. It will be seen that the diffraction patterns of all the samples, except that of plaster of Paris, are identical. This indicates the instability of the alleged rhombic form of gypsum¹ and shows that the presence of electrolytes during the precipitation does not modify appreciably their composition or structure.

¹ Cf. DAVIS, *J. Soc. Chem. Ind.*, **26**, 727 (1907).

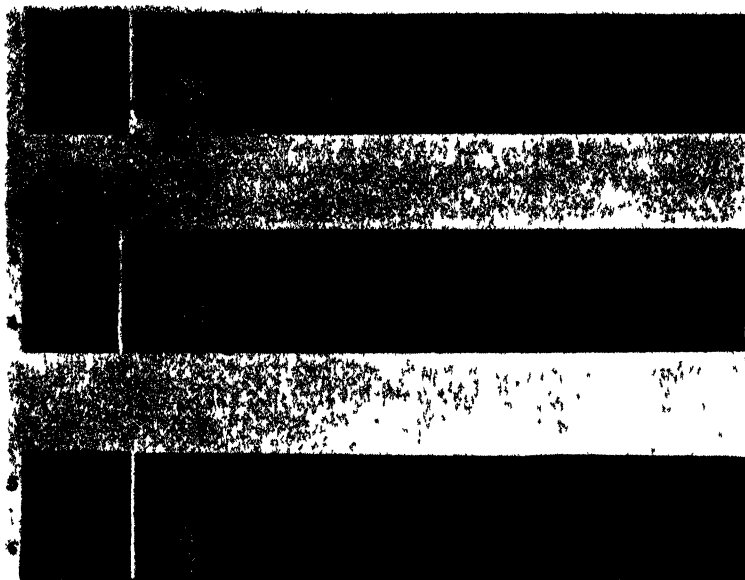


FIG. 15

X-Ray Diffraction Patterns of (1) Plaster of Paris and of the Products formed by the Action of Plaster with (2) H_2O after 1 month (3) H_2O after 1 hour (4) $0.5\ N\ NH_4C_2H_3O_2$ (5) $10\ N\ NH_4NO_3$ (6) $7\ N\ (NH_4)_2SO_4$.

Effect of adding Gypsum Nuclei to Plaster of Paris on the Rate of Set in Solutions of Foreign Electrolytes

Since the slowness of set of plaster in dilute ammonium acetate solutions is not due to the formation of a gypsum of different crystal structure, an attempt was made to determine whether the retarding was due primarily to the slowness of formation of nuclei or to the failure to precipitate even in the presence of nuclei. Hansen's observations with $N/10\ NH_4C_2H_3O_2$ indicate that the first factor is important since he found that the time for attaining the maximum temperature was decreased from approximately 85 minutes to 55 minutes by shaking the acetate solutions for 35 minutes with a few grams of plaster of Paris before mixing it with the bulk of the sample. This increase in rate is not as large as one might expect from the observed effect of nuclei in the absence of electrolytes as shown in Fig. 2 of this paper. This could be due either to the formation of relatively few gypsum nuclei by only 35 minutes' action of $N/10$ acetate solution or to relative slow growth on the nuclei. To determine, if possible, what is the effect of added nuclei, the time-temperature curves were obtained for pastes prepared by mixing 35 cc of $N/10\ NH_4C_2H_3O_2$ with 50 grams of plaster to which was added (1) no gypsum (2) 0.5 gram of gypsum formed by the action of water on hemihydrate, dried and ground and (3) 0.5 gram of gypsum formed by the action of $N/10$ ammonium acetate solution on hemihydrate, washed, dried, and ground (4) same as 3 except that the gypsum

sample was not washed, dried, or ground. The latter sample was prepared by adding plaster to the acetate solution and stirring gently with a current of air for several hours. The dried gypsum which passed a 100-mesh sieve was mixed thoroughly with the plaster before adding the electrolyte while the moist gypsum was suspended in the electrolyte before mixing with the plaster. The observations were repeated using $N/2$ $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ instead of the $N/10$ solution. The results are summarized in Table X. The time-temperature curves for the various seeded samples with $N/2$ $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ are reproduced in Fig. 16.

TABLE X

Effect of Seeding with Gypsum on the Rate of Set of Plaster of Paris—
Electrolyte Mixtures

Electrolyte added 35 cc	50 g Plaster of Paris seeded with	Time for maximum temperature
0.1 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$	0	83
0.1 N "	0.5 g gypsum formed in H_2O , dried, and ground	19
0.1 N "	0.5 g gypsum formed in 0.1 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$, washed, dried and ground	19
0.1 N "	0.5 g gypsum formed in 0.1 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ not washed, dried and ground	68
0.5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$	0	240
0.5 N "	0.5 g gypsum formed in H_2O , dried and ground	43
0.5 N "	0.5 g gypsum formed in 0.5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$, washed, dried and ground	55
0.5 N "	0.5 g gypsum formed in 0.5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$, not washed, dried and ground	206
10 N NH_4NO_3	0	121
10 N "	0.5 g gypsum formed in H_2O , dried and ground	37

A reasonable explanation of the cause of the slowness of set of plaster in ammonium acetate solutions is furnished by these data. Comparing curve 1 with curve 4 of Fig. 16 it is evident that the addition of 1 percent of finely ground gypsum, formed in water, to plaster cuts down enormously the inhibition period, and decreases the time for reaching the maximum temperature from 240 minutes to 43 minutes. This means that the supersaturation is maintained sufficiently great that the growth on the added nuclei is fairly rapid. On the other hand, if the plaster is seeded by the same amount of the fine crystals formed in 0.5 N acetate and not washed, dried, and ground, the

rate is but little faster than if no nuclei at all are added (compare curves 3 and 4, Fig. 16). Evidently gypsum formed in the presence of acetate does not furnish satisfactory nuclei for starting the growth of the crystals; hence the precipitation from the supersaturated solution is greatly retarded. Since the crystal lattice is the same in gypsum formed in water as in gypsum formed in the acetate solution it is probable that the failure of the latter to act as effective nuclei is due to a film of acetate adsorbed on all the faces of the crystals. In support of this view it was found that washing, drying and grinding the

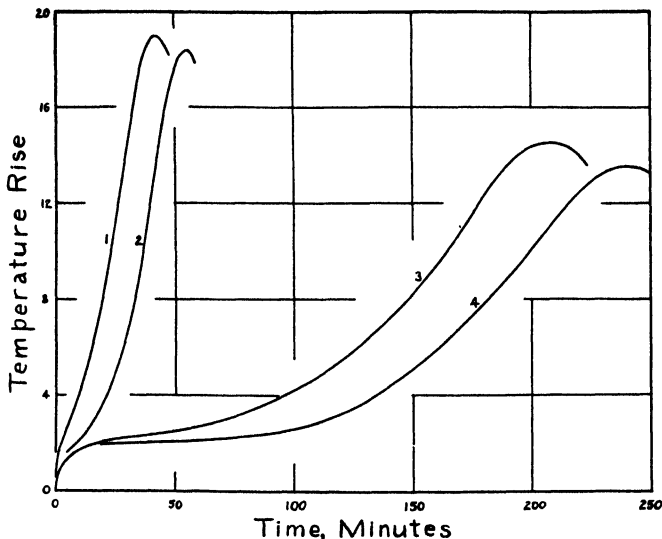


FIG. 16

Time-Temperature Curves obtained with Plaster of Paris in 0.5 N $\text{HN}_4\text{C}_2\text{H}_3\text{O}_2$ Solution Seeded with Gypsum Crystals formed in (1) H_2O (2) 0.5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ Solution, washed, dried, and ground (3) 0.5 N $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ Solution not washed, dried, or ground (4) Not Seeded at all.

crystals formed in the presence of acetate gave effective nuclei and so accelerated the rate of set almost as greatly as the nuclei formed in water (compare curves 1 and 2 of Fig. 16).

The ultimate effect of ammonium acetate on the rate of set of plaster of Paris appears to be similar to that of small amounts of glue and gelatine, the adsorption of which on the gypsum nuclei inhibits or prevents the growth of the crystals and so delays or prevents the setting of the plaster.¹

The addition of nuclei to the 10 N NH_4NO_3 plaster paste likewise speeds up the initial rate of reaction by cutting down the inhibition period and by furnishing a large number of centers of precipitation. (See Fig. 17.) Because of the low supersaturation, however, the reaction is slow in reaching completion even in the presence of added nuclei, as evidenced by the unusually broad portion in the region of maximum temperature in the time-temperature curve.

¹ Rohland: *Z. anorg. Chem.*, **40**, 182 (1904); Traube: *Kolloid-Z.*, **25**, 62 (1919); Ostwald and Wolski: **27**, 78 (1920); Neville: *J. Phys. Chem.*, **30**, 1037 (1926).

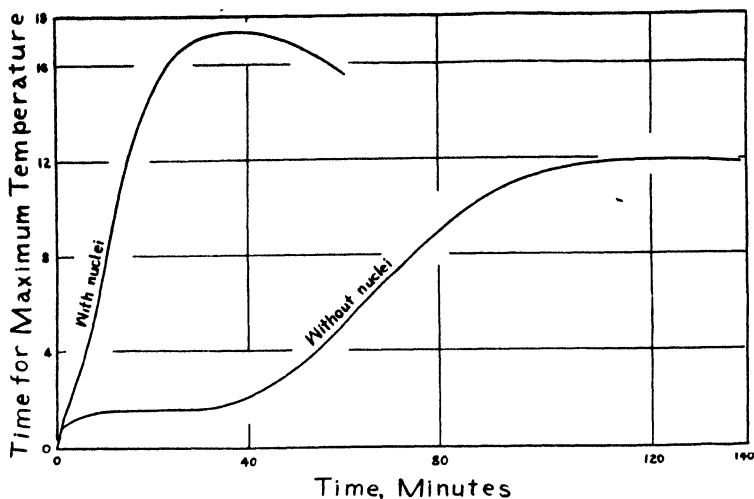


FIG. 17

Time-Temperatures Curves obtained with Plaster of Paris in 10 N NH_4NO_3 Solutions with and without the Addition of Gypsum Nuclei.

Summary and Conclusions

The results of these investigations are as follows:

1. Thermometric and optical observations of the hydration of plaster of Paris were made with the end in view: (1) of throwing light on the existence or non-existence of gel formation as a stage in the setting process; and (2) of formulating a general theory of the effect of foreign electrolytes on the rate of set.

2. A survey of the experimental evidence in support of the hypothesis that a jelly is formed before the interlacing mass of gypsum crystals is laid down, discloses the absence of any direct evidence of jelly formation. The indirect evidence is from two main sources: (1) the observation that gypsum can be thrown down under certain conditions as a gelatinous precipitate (Cavazzi, Neuberg) and as a jelly (Baykoff); and (2) the existence of a period of inhibition in the time-temperature curve which Neville attributes to the formation of a gel or adsorption complex between the plaster and water, a process accompanied by but little heat effect; and which Budnikoff attributes to the formation of a gel around the plaster particles protecting them from the action of water until crystallization of the gel takes place.

3. Baykoff's gypsum jelly formed by shaking 10 percent $(\text{NH}_4)_2\text{SO}_4$ solution with an excess of plaster for 2 minutes and allowing to stand, is a "false gel" consisting of a mass of relatively large interlacing crystals of gypsum which have entangled water. The only point of resemblance between the cloudy, non-uniform, entangling mass of crystal needles and a silica jelly is that both stay in the containing vessel when the latter is inverted.

4. The period of inhibition is not due to the formation of an adsorption complex between water and plaster "whereby the two reactants are brought

into chemical contact" (Neville); but is the result of delayed precipitation from a supersaturated solution owing to dearth of gypsum nuclei. The period of inhibition can be completely eliminated by the addition of less than 1 per cent of finely powdered gypsum to the plaster.

5. When the time to attain the maximum temperature is plotted against the weight of finely powdered gypsum added to the plaster, a parabolic curve is obtained. By using the equation for this curve the initial amount of gypsum nuclei in a slow-setting plaster can be calculated. A sample containing less than 0.1 milligram of gypsum in 50 grams required 100 minutes to attain the maximum temperature on mixing with 35 grams of water while a sample containing 0.5 gram of gypsum in 50 grams attained the maximum temperature in 14 minutes. The tensile strength of the two samples of set plaster was approximately the same.

6. Suitable ignition to eliminate gypsum nuclei is all that is necessary to obtain a relatively slow setting plaster of Paris and the rate of set can be increased to any desired point by seeding with a suitable amount of finely powdered gypsum.

7. Rapid stirring of a plaster of Paris-water mixture increases the rate of set by breaking down the supersaturated solution of gypsum supplying nuclei and thereby decreasing the length of the induction period. When time of stirring is plotted against time for attaining the maximum temperature, a parabolic curve results similar to that obtained by the direct addition of varying amounts of gypsum to the plaster. (See 5 above.)

8. Since the setting of plaster of Paris involves the precipitation of gypsum from its supersaturated solution (Lavoisier, LeChâtelier) the effect of foreign electrolytes on the process may be considered in the light of von Weimarn's theory of the rate of precipitation of nuclei and of the Nernst-Noyes equation for the rate of growth on nuclei as a result of diffusion. In general, the initial rate of formation of nuclei is proportional to $(Q-L)/L$ where Q is the total concentration of the substance which is to precipitate and L the solubility. $(Q-L)/L = P/L$ is the percentage supersaturation. The velocity of growth on the crystal nuclei, under otherwise constant conditions, is determined by $Q - L$, the absolute supersaturation.

9. The ratio of the solubility of plaster of Paris to that of gypsum in water at 25° is 4.5. If the saturation concentration of plaster of Paris hydrates to gypsum without precipitation, the percentage supersaturation is 3.5. Because of the relatively long inhibition period with pure plaster, this percentage supersaturation is insufficient to cause rapid initial precipitation of nuclei which must be present for a rapid reaction to take place throughout the mass of the plaster.

10. If the addition of a foreign electrolyte to water cuts down the period of inhibition, it follows in accord with von Weimarn's theory that the initial percentage supersaturation of the solution with respect to gypsum must be definitely increased. This may be accomplished in one of two ways: either the solubility of the hemihydrate is increased appreciably more than that of

gypsum in the presence of the foreign electrolyte or the solubility of the gypsum is decreased appreciably more than that of hemihydrate by the presence of the foreign electrolyte. In the first case P/L is increased because $Q - L = P$ is increased proportionately more than L ; and in the second case P/L is increased because L is decreased proportionately more than P . Conversely, if the addition of a foreign electrolyte to water lengthens the inhibition period the initial percentage supersaturation of the solution with respect to gypsum should be decreased.

11. In general, for a foreign electrolyte to change the initial rate of formation of nuclei and hence the rate of set as compared to the rate in water alone, all that is necessary, other things being equal, is for the ratio of the solubility of hemihydrate to the solubility of gypsum to be greater or less than 4.5, the ratio of the solubilities in pure water.

12. The form of the gypsum crystals in the set plaster is influenced in great measure by the factors determining the absolute supersaturation and the number of nuclei. If L is small so that the $Q - L$ value is relatively large, the crystals grow very rapidly and are not well formed; while if L is large so that $Q - L$ is relatively small, large well-formed crystals result, provided the plaster is not seeded with gypsum nuclei.

13. The above deductions have been confirmed by extended thermometric and microscopic observations on the setting of plaster of Paris in solutions of varying concentrations of NH_4NO_3 , NH_4Cl , NH_4CNS , $(\text{NH}_4)_2\text{SO}_4$, NaCl and MgCl_2 .

14. Contrary to what would be predicted from von Weimarn's theory the rate of set of plaster of Paris in dilute $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ solutions is much slower than would be expected from solubility relations and the prevailing supersaturation. The reason for the retarding action of $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ is that gypsum does not deposit readily from its supersaturated solution on gypsum nuclei formed in the presence of $\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$ probably because of an adsorbed film of acetate on all the surfaces of the crystals. The addition of gypsum nuclei formed in the absence of acetate, induce a rapid rate of set.

15. The effect of such salts as ammonium acetate and ammonium citrate on the rate of set of plaster of Paris is similar to that of small amounts of glue and gelatin, the adsorption of which on the gypsum nuclei inhibits or prevents the growth of the crystals and so delays or prevents the setting of the plaster.

16. X-radiograms of the widely different forms of crystals obtained by the actions of plaster in solutions of the several electrolytes, disclose that the crystals are identical in structure with gypsum formed in the presence of H_2O alone.

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THE KELLY TUBE AND THE SEDIMENTATION OF PORTLAND CEMENT

BY CHARLES G. DUNCOMBE AND JAMES R. WITHROW

Introduction

This paper, after reporting the results of work done on the sedimentation of cement samples, using Kelly's sedimentation tube, describes a newly developed apparatus used in obtaining rates of settling of portland cement.

It is well known that there are a large number of pulverized products or fine powders in commercial production at the present time and that the degree of fineness is of great interest to many manufacturers and consumers of materials. Cements, pigments, and rubber fillers are a few examples of such materials. It is almost as well known that there is no method for the determination of fineness available for use in control laboratories with complete satisfaction. The control laboratory desires a method which may be carried out by the average laboratory worker with but little preliminary training and, in addition, that the method be carried out without the use of expensive or complicated apparatus. Finally, the method should permit the evaluation of fineness in at least two hours from the time that the sample is received from the grinding department or the receiving platform, as the case may be.

The well known and easily operated screening test meets the requirements of the control laboratory in these respects but cannot be used successfully for sizes much smaller than 200 mesh because of variations in size of wire and mesh and because of difficulties in obtaining peptization or preventing agglomeration.

Disregarding requirements of the control laboratory, there are several methods for the determination of the fineness of powders which have been largely developed in the field of colloid chemistry. In general these methods are (1) microscopic measuring and counting, (2) elutriation followed by microscopic measuring and counting, and (3) sedimentation methods.

This paper describes work done on one form of sedimentation method, using cement as the powder to be studied. We have worked on cement because of interest in cement production problems. Sedimentation analysis was selected for investigation because it was felt that this group of methods offered opportunities for the development of a control method. From the group of methods based on separation followed by microscopic measuring and counting, the air analyzer has already developed into a tool in use to some extent in control laboratories. This analyzer, developed by Pearson and

Sligh¹ will, according to Gonnerman² produce a single separation in from 60 to 100 minutes and, by repeating the separations with changes in the apparatus or conditions, as many separations as desired to show the fineness distribution may be obtained.

The microscopic methods have been reviewed by Work.³ It does not seem likely that the use of the microscope will ever be popular in control laboratories for determining fineness since such use would involve not only the possession of a microscope but also the employment of a worker skilled in its use.

Literature

A review of sedimentation methods and a mathematical analysis of these methods has been contributed by Odén.⁴

The sedimentation methods based on a study of uniform suspensions can, according to Odén, be subjected to mathematical analysis under one of the following heads:

1. The variation of the specific gravity and the concentration with time at a definite distance below the surface of the suspension.
2. The variation of the specific gravity and the concentration with distance from the surface at a definite time.
3. The variation of the hydrostatic pressure with the time at a definite distance from the surface.
4. The variation in weight on an immersed body with time.
5. The variation in hydrostatic pressure with distance from the surface at a definite time.
6. Accumulation of the particles on the bottom as a function of time.

Odén also describes methods of Audubach and of Werner where uniform suspensions were not used but the dispersed sample was placed at the top of a column of fluid and the rate of settling observed. References to further use of these methods could not be found in the literature and it appears that they have not come into established use.

With the exception of the methods of Audubach and Werner, all of the methods of sedimentation analysis described by Odén depend on studying the rate of change of some property of a uniform suspension either with respect to time or with respect to distance from the surface after a definite time has elapsed. Special methods of treatment of the observed data are necessary in order to obtain the particle size distribution of the sample. It is not the purpose of this paper to discuss or consider these special methods of treatment and references regarding them are, therefore, not made.

¹ J. C. Pearson and W. H. Sligh. An Air Analyzer for determining the Fineness of Cement. Technical Paper No. 48. U. S. Bureau of Standards, Washington, D. C., September 8th, 1915.

² H. F. Gonnerman, Manager of Laboratory, Portland Cement Association. Private Communication to James R. Withrow, July 10th, 1929.

³ Lincoln T. Work. "The Graphical Analysis of Distribution Curves for Pulverized Materials." Ph.D. Dissertation, Columbia University, New York City, (1928).

⁴ Jerome Alexander: "Colloid Chemistry," pp. 861 et seq. (1926).

After some examination of the literature, it appeared that the methods of the third group were the simplest and required less apparatus than any other group, with the possible exception of the hydrometer method. Accordingly, attention was concentrated on the literature of the third group, namely the variation of the hydrostatic pressure with time at a definite distance from the surface of the suspension.

Wiegner's apparatus, which was the first to be described in which the rate of change of the hydrostatic head at a certain distance below the level of the

suspension was measured with respect to time, consisted of a tube similar to that shown in Fig. 1. Ostwald and von Hahn modified this tube by placing the stop cock at the top of the tube of small diameter, hereafter referred to as

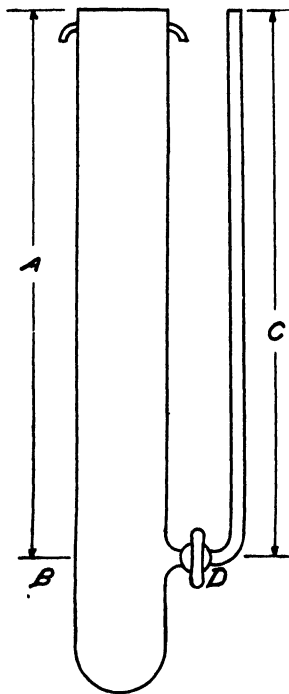


FIG. 1

Wiegner's Sedimentation Tube

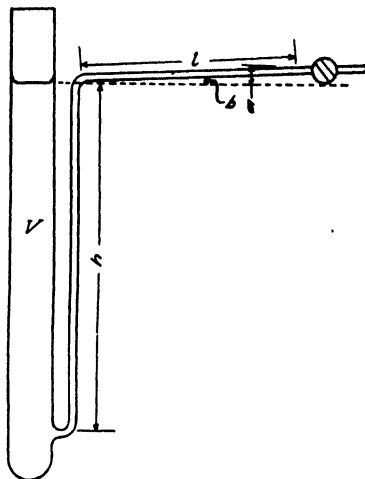


FIG. 2

Kelly's Original Sedimentation Tube

the side tube. Gessner and Wiegner mounted a light source in front of the side tube and a camera, containing a sensitized paper mounted on a moving drum, behind the side tube and obtained a photographic image showing the fall curve directly. All of these workers employed relatively concentrated suspensions, often containing as much as 50 grams per liter of the solid. Lorenz, who employed essentially the photographic apparatus of Gessner, recommended a concentration of from 2-4%.

Kelly¹ recognized that the use of concentrated suspensions was not desirable and described an apparatus, which he developed so as to permit the use of more dilute suspensions. Kelly's original tube is shown in Fig. 2.

¹ Ind. Eng. Chem., 16, 928 (1924); Colloid Symposium Monograph, 2, 29 (1925).

As a result of experimental work, he found that the rate of evaporation from the side tube which he used was a source of error and he proposed a modified form of the tube which is shown in Fig. 3. This tube, according to Kelly, had not been built when his article was written.

That class of sedimentation method in which the variation in hydrostatic pressure at a fixed point below the level of the suspension is observed as settling proceeds, appeared to be worth further investigation as the source of a method which might be suitable for control purposes. Of the apparatus of this type, the tube of Kelly appeared at first sight to be sufficiently simple and to possess advantages over other tubes of this class. Consequently, it was decided to utilize this tube for the first stages of the work which was to carry out sedimentation of cement samples, to make a study of the accuracy and convenience of the method, and to improve the accuracy and convenience wherever possible or desirable.

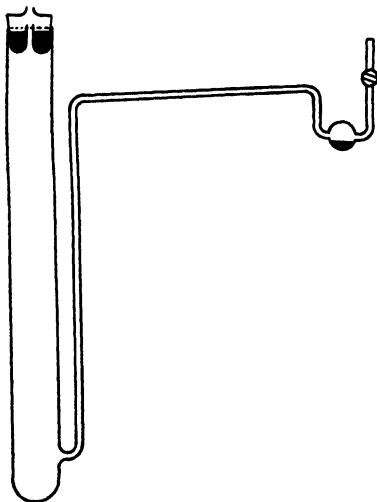


FIG. 3
Kelly's Proposed Sedimentation Tube

I. Experimental Work with the Kelly Tube

The Kelly Equation. Kelly derived a formula for use with his tube and in the derivation the assumption is made that the height of the suspension remains constant throughout a sedimentation.

It appears that this may not be strictly true and that error may be introduced by this assumption. Accordingly the formula was, first of all, subjected to critical examination.

Kelly developed his formula by setting the hydrostatic pressure of the suspension equal to the hydrostatic pressure of the clear liquid in the side tube at the junction of the side tube to the main tube which contains the suspension. Thus, referring to Fig. 2,

$$D h = d (a + h) \quad (1)$$

where D is the density of the suspension and d is the density of the clear liquid in the side tube (and also the suspending liquid). D is equal to

$$\frac{(V - v)d + w}{V} \quad (2)$$

where V is the volume of the suspension above the entrance to the side tube and v is the volume of the pigment in suspension whose weight is w . Also

$$a = l \sin b \quad (3)$$

where b is the angle which the horizontal portion of the side tube makes with the exact horizontal. Substituting equations (2) and (3) in equation (1) and

transposing, Kelly arrives at the following expression for the weight in suspension:

$$w = \frac{dVS l \sin b}{h(S-d)} \quad (4)$$

where S is the specific gravity of the pigment in suspension. According to Kelly, since w and l are the only variables for a given experiment, equation (4) reduces to the form

$$w = K l \quad (4a)$$

in which form it is conveniently used.

Although Kelly did not go into this, for evaluation of the distribution of fineness it is convenient to obtain the weight settled. Kelly's formula (4) is easily extended to this form by remembering that the weight settled equals the weight in suspension at the start minus the weight in suspension at the time in question, or

$$\text{weight settled} = \frac{dVS \sin b (l_0 - l_t)}{h(S-d)} \quad (5)$$

where l_0 is the reading at zero time and l_t is the reading of the manometer side tube at the time in question.

The formula of Kelly is developed under the assumption that "h," the height of the suspension remains constant. It does not appear that this is strictly true for, as the solid settles out, the meniscus in the side tube recedes and, in so doing, transfers a quantity of liquid to the main tube, thus increasing the height of the suspension. For one centimeter of travel by the meniscus there will be a definite increase in the height of the suspension depending first on the volume of liquid in the side tube per centimeter of length and second on the centimeters of height of liquid in the main tube per cubic centimeter. The product of these two values is the increase in height in the vertical tube for one centimeter of travel of the meniscus along the side tube.

Thus, in Kelly's original equation (1), the term "h" should be replaced by the term " $h + \Delta h$," where "h" is the height of the suspension at the start of the experiment. Accordingly, we have

$$D(h + \Delta h) = (a + h) d \quad (6)$$

Repeating the development as before and neglecting the effect of the liquid flowing into the vertical tube from the side tube on the density of the suspension, we obtain for the weight in suspension

$$w = \frac{(l \sin b - \Delta h) d VS}{h(S-d)} \quad (7)$$

Representing the increase in height in the vertical tube caused by the recession of the meniscus over a distance of one centimeter by the term C_1 is permissible, since, for a given tube, this is constant. Also the term " $\sin b$ " may be represented by C_2 since it is also constant for a given tube. Thus we obtain (for the weight settled)

$$w = \frac{dVS (l_0 - l_t) (C_1 + C_2)}{h(S - D)} \quad (8)$$

$$\text{or} \quad w = K''(l_0 - l_t) \quad (9)$$

Effect of Tube Dimensions on Equation Error. The amount of error which is introduced by assuming h to be constant may be calculated by assuming that formula (8) is correct. Comparing this with equation (5), the error is proportional to the term C_1 and the percent error is given by the expression

$$\frac{C_1}{C_1 + C_2} \times 100 \quad (10)$$

In the tube suggested by Kelly in which he recommended an angle of inclination of $1^\circ 30'$ and a bore of 2 mm. for the side tube and a diameter of 2 cms. for the vertical tube, C_1 takes the value of 0.01 and C_2 the value of 0.0262 from which the percent error would be 28 percent.

With the Kelly tube used in this work, to be described later, the sine of the angle or C_2 was 0.02. The diameter of the side tube was 2.5 mm. and 135.5 ccs. were contained in 34 cms. of height in the vertical tube. C_1 was thus 0.012 from which the percent error was $37\frac{1}{2}$ percent.

In a tube constructed according to the idea of Kelly, described by Mack and France¹ 2.38 centimeters of horizontal side tube contained 0.1 cc. and 25.2 centimeters of height in the vertical tube contained 99 ccs. Thus C_1 was 0.01. C_2 was given as 0.03. Therefore, in this tube the error in weight settled amounts to 25 percent.²

For solids which settle out completely and a reading thus obtained for the completely settled condition, the final reading may be designated by l_∞ and the total weight settled becomes, from equation (8)

$$w_\infty = \frac{dVS (l_0 - l_\infty)}{h(S - d)} (C_1 + C_2) \quad (11)$$

Since percent settled at any time is the ratio of the weight settled at that time compared to the total weight settled and multiplied by 100, or in the ratio of equation (8) to equation (11) all of the constants vanish and the expression reduces to the form

$$\frac{l_0 - l_t}{l_0 - l_\infty} \times 100 \quad (12)$$

¹ "A Laboratory Manual of Elementary Physical Chemistry," 182 (1928).

² During discussion of the paper, R. Bradfield questioned the validity of all sedimentation methods because Keen and Crowther have shown that, in the case of the Kelly tube, when the clear liquid enters the main tube from the side tube, it does not displace the suspension upward but travels up the side of the tube and, in so doing, sets up eddy currents and that these eddy currents introduce errors which are much larger than that caused by neglecting the increase in height of the suspension in determining the weight of solid in suspension or settled out. This objection may have some weight in the case of the Kelly tube. We have no experimental data on it. However, the modified apparatus described later in the paper, transferred only about 0.0012 cc. of liquid from the side tube to the main tube over a period of several days. It would appear that such eddy currents, if produced at all, must be very slight and of small effect, in this case.

From this it is evident that, if percent settled is calculated directly from the readings, using equation (12), all constants of the Kelly formula vanish and they need not be determined at all. In addition, one step of the calculation is eliminated, since the results must be calculated to percent settled in any event, in order to be susceptible to the special treatment by which distribution data is obtained.

However, for another purpose, the evaluation of the Stokes equation, it is necessary to determine the density and viscosity of the liquid and the density of the solid as well as the height of the suspension above the entrance to the side tube.

Apparatus. The first sedimentation tubes constructed for use in this work were similar to that illustrated in Fig. 3. However, the small bulbs located at the outer end of the side tube were a source of trouble. They rendered the tubes more difficult to clean and dry and, when the bulbs were charged with liquid preliminary to starting a run, careful handling was required to prevent transferring some of the liquid to a portion of the capillary directly adjacent to the bulb where a short column or meniscus of the liquid would be formed. After liquid had been placed in the bulb, the side tube had to be filled with clear liquid and then excess of clear liquid poured out of the

vertical tube and here accidents were frequent. Finally, the extremely rapid reaction of settling required considerable speed in starting and prevented careful and fine adjustment of the level of the suspension and, as a result of this, often the level would be too high, forcing the side tube liquid into the bulb and requiring complete emptying and restarting of the run. Consequently, the bulbs were eliminated from this position. Experimental work showed that some form of bulb was absolutely necessary and therefore the bulbs were constructed separately and mounted on the end of the side tube by means of glass tubing and mercury seals. The protection bulb finally used for the large tube was also separately constructed and was attached by means of a cork covered on the inside with tin foil and sodium silicate. The most satisfactory scale was obtained by lashing a thermometer to the horizontal portion of the side tube. Fig. 4 shows the complete tube used in the latter runs. Two of these improved tubes were constructed, whose dimensions are given in Table I.

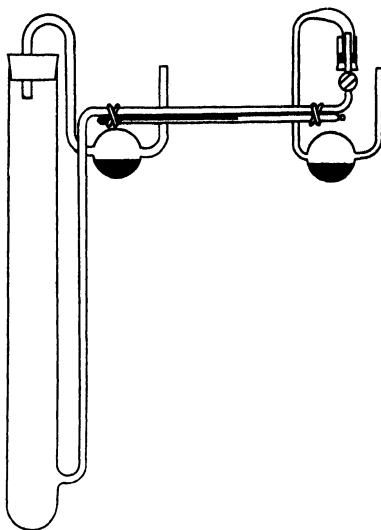


FIG. 4
Modified Form of Kelly Tube

TABLE I
Dimensions of Kelly Sedimentation Tubes

	Tube 1	Tube 2
Total height, vertical tube	47 cms.	47 cms.
Inside diameter, vertical tube	23 mm.	23 mm.
Inside diameter, manometer tube	2.5 mm.	2.5 mm.
Length of scale, manometer tube	35 cms.	34 cms.
Height of manometer scale above side tube connection	34 cms.	32 cms.
Height of side tube connection above bottom of large tube	6 cms.	5.5 cms.

Procedure. In the first experiments, efforts were made to use a definite weight of sample with a view to obtaining some confirmation of the improved formula already developed (equation 8). This involved the preparation of a suspension having less volume than actually required and complete transfer of this suspension to the tube with subsequent addition of sufficient clear liquid to adjust the level to the desired place. This, in turn, required mixing the suspension in the tube before starting a run. After several experiments, this procedure was abandoned and an excess of suspension was prepared from which the tube was filled as rapidly as possible.

In the selection of the details of the procedure, it is to be recognized that cement is a material which cannot be subjected to sedimentation analysis in water or an aqueous solution because the action of the water on the cement would alter the condition of the particles under investigation. Even the use of substances in which water is an impurity such as glycerin, alcohol, etc., can only be used after an investigation shows that cement cannot remove water from such materials. Mineral oil, after refining, is probably less liable to contain water than the majority of liquids. Therefore we arbitrarily elected to use stanolind, a water-white refined mineral oil of high viscosity, and to mix this with kerosene in proportions to produce a liquid mixture of the desired viscosity.

It was found that when a high viscosity liquid, produced by using 65 percent stanolind and 35 percent kerosene, was used in the side tube, approximately 20 minutes was required for the meniscus to come to equilibrium after a small disturbance. This lag is fatal to sedimentation and shows that a liquid of low viscosity must be used in the side tube. Theoretical considerations show that a combination of two liquids of different viscosity and density one in the side tube and the other in the vertical tube can be used without complicating the calculations or invalidating the results, if the readings are calculated directly to percent settled. There must be no interchange of the two liquids in the side tube although the flow of the manometer tube liquid into the vertical tube does no harm, except possibly to create eddy currents. Toluol was selected as the liquid to be used in the side tube.

Measurements showed that 134 ccs. were contained in each of the sedimentation tubes when filled to the proper level for starting. Arbitrarily, it was

decided to use 1.5 grams of cement in 150 cc. of oil, or a 1 percent suspension. It was found that, by heating the oil containing the sample, apparently good peptization was obtained. Since the character of the oil might conceivably change somewhat by this heating, viscosity and density measurements were made on samples of clear oil after each sedimentation had been completed.

Experiments showed that, when the wall of the vertical tube was wetted with the suspension above the level of the suspension during the course of filling, it required ten minutes for this liquid to drain down from the walls and as it drained down it increased the height of the suspension, thereby introducing an error which distorted the starting readings.

In the early runs, attempts were made to add the suspension to a definite mark on the vertical tube so located that, when the stop cock was opened, the meniscus in the side tube would arrive at a position near the outer end of the horizontal portion of the side tube. Experience showed that it was very difficult to do this, and in later runs, as soon as the stop cock was opened, a final rapid adjustment of the level of the suspension was made by means of a pipette. For the last few runs, in which protection bulbs were used over the large tubes, these bulbs were not affixed until the level had been adjusted.

As soon as the suspension had been added, a stop watch was started and readings of the position of the meniscus were thereafter taken with respect to time. Since it was impossible experimentally to obtain a zero reading, this was obtained by plotting the first few readings on an auxiliary curve and extrapolating back to zero time. The readings were calculated to percent settled as already indicated, namely, by taking the difference between the zero reading and the final reading as 100 percent settled and the difference between the zero reading and the reading at the time in question as proportional to the percent settled at that time.

Experimental Results. The experimental work covered a series of thirty runs. Of these the results of the last two runs were of most significance. The readings for these runs, quite similar to others, are shown in Table II, the readings for the early portions of the runs being omitted for the sake of brevity.

In neither of these runs did the readings ever become constant.

In other runs, however, the readings became constant after 20 or 21 days. In all of these runs, the liquid in the main tube appeared clear after approximately one week.

In several runs, the readings became constant while the suspension was still turbid, i.e., before settling was complete. In one, a tiny droplet of water was found in the side tube but in the others, no reason for the stationary meniscus could be found.

In the early runs, entirely too many readings had to be ignored in drawing smooth curves, evidently due to fluctuations in the thermostat bath temperature, due to inadequate stirring. When the rate of stirring was increased, these variations were reduced.

The presence of lag was shown in preliminary experiments when a liquid of high viscosity (about 0.2 poise) was used in the side tube. This lag was

TABLE II
Rate of Settling, Cement Sample No. 2

Run 29		Run 30	
Time	Reading	Time	Reading
—	—	—	—
—	—	—	—
420 min.	82.4	315 min.	127.2
455	81.0	360	121.8
500	79.0	1135	115.0
19 hr. 15 min.	57.6	19 hr. 20 min.	113.5
22 15	55.4	21 35	112.4
24	53.6	23 25	111.8
25 45	51.8	25 35	111.0
28	50.5	41	105.5
43 25	45.5	49 25	103.7
52	43.7	70 15	100.3
72 40	40.2	113 20	95.0
115 45	36.0	122	94.0
124 15	34.9	140 10	92.8
142 35	33.6	8 days	89.7
8 days	29.3	12	86.2
12	24.0	15	83.9
15	20.1	19	83.0
19	17.0	20	82.5
20	15.8	22	81.5
22	13.0	24	79.5
24	11.4	26	78.0
26	10.0	28	77.0
28	8.0	31	76.0
31	5.5	35	74.5
35	2.0	40	72.2
40	-2.0	44	70.6
44	-5.0	48	67.9
48	-7.5	55	64.9
55	-11.0	57	64.0
		59	60.5

Readings stopped because meniscus went off scale. Side tube bulb still contained toluol.

After last reading, tube was removed
No toluol was left in side tube bulb.

found to be approximately 20 minutes, i.e., when, after equilibrium had been obtained for the meniscus, the level was disturbed temporarily so as to displace the meniscus approximately 2 centimeters, it required approximately 20 minutes to return to nearly the same position or rather to a position of rest. The meniscus never did return to exactly the same position.

Several runs were deliberately extended only through the early stages of settling in order to study the conditions at the start of the run. Suspicion was aroused that a different shaped curve was obtained at the start of a run when the meniscus attained its position of dynamic equilibrium by a continuous and uninterrupted recession, than when it rose and then began to recede. These two types of start were the result of the method of adjusting the level of the suspension. Two runs were started with a falling meniscus, (uninterrupted recession) and two runs were started with arrested meniscus (first rising and then falling). When the readings were plotted in curve form, it was seen that, when the meniscus fell without interruption, the slope of the curve for the first ten minutes was steeper than when the meniscus has been arrested.

Five runs were regarded as completed runs when they were made and the percent settled was calculated. Table III shows some of the values from these runs.

TABLE III
Percent settled with Respect to Time

Time (min.)	Cement 1		Cement 2		
	Run 5	Run 6	Run 9	Run 15	Run 16
5	10.28	12.7	9.2	8.7	10.37
10	18.14	21.5	17.1	14.8	19.45
20	28.93	33.3	27.5	23.7	30.1
30	35.53	40.5	34.5	31.2	37.8
40	40.86	45.7	40.1	36.3	43.6
50	44.79	49.7	44.6	—	46.6
60	48.47	53.1	48.5		50.0

Runs 5 and 6 are directly comparable, being made in the same tube, at the same temperature, with the same height of suspension and using the same mixture of sedimentation oil. The three runs for cement 2 are only comparable within about 2 percent, since there was small differences in the height of the suspension between the three runs.

Discussion of Results. Run 29 and 30 showed that, even under the best conditions, using only glass and mercury seals and locating the bulbs of Kelly at approximately the same level as the liquid levels in the vertical tube and the side tube, respectively, evaporation continued to take place and a stationary position of the meniscus was never obtained. Other runs also showed this same behavior. This confirmed Kelly's observation on the run reported by him (a short one of approximately 8 hours) for, with longer runs than reported by Kelly, we find this error intensified.

Knowing that evaporation takes place, it is possible to explain the unusually long time required for a constant reading to be obtained in some of the runs. The fact that the meniscus ever became stationary in these runs must have been due to trouble such as was encountered in other runs where the meniscus became stationary before settling was complete.

It is apparent that the method used to obtain percent settled values depending, as it does, upon the obtaining of a final constant reading, cannot be directly used, if evaporation takes place. It is possible to prolong a run and perhaps obtain a constant rate of fall of the meniscus which would be due to evaporation alone and then to apply this evaporation correction to all readings taken. Such procedure would be entirely too laborious and time consuming to be of much value, if any other method can be made to serve.

Results of all runs made, therefore, whether initially regarded as successfully completed or not, must be regarded as affected with this evaporation error.

The percent settled values of the five runs cited cannot be considered as reliable because of evaporation loss. The fact that constant readings were ever obtained must have been due to some interfering substance or situation which caused the meniscus to become stationary. This could have happened at different relative times in the history of the runs and thereby affected the percent settled values. The evaporation loss did not affect the relative values of percent settled in the early history of the run and comparisons could be roughly made, except for the possibility of the meniscus having been arrested.

There is also the possibility that complete peptization was not obtained. There is no evidence to show that any of the samples were completely peptized. This does not, however, invalidate any of the results bearing on the operation or usefulness of the apparatus. Lack of peptization merely means larger particles.

In connection with the runs it is important to note that the method for calculating percent settled depends on extrapolation backwards to obtain a value for the reading at zero time. The total change in readings and therefore the value for 100 percent settled depends, in part, on the zero reading and, if this is incorrect, all percent settled values are in error as a result. To illustrate this, suppose that a total difference in readings of 150 was obtained but that the zero reading was 10 too low, in other words the correct difference in reading should be 160. Then at some intermediate reading, say for 50 percent apparently settled, the correct percent settled would be $85/160$ instead of $75/150$ or 53 percent. This is an error (3 percent in the example) which cannot be permitted to exist if it is possible to eliminate it.

In discussing the results, it should be stated that it was to be expected that troubles would be experienced traceable to the type of powder under examination. We are dealing with a powder which, from the screen analysis, is known to have about 10 percent residue on 200-mesh screen. It has been the object in this work to evaluate the coarse particles as well as the finer portion by sedimentation analysis.

In later work, to be described in another paper, it was found that the coarsest particles had a dimension of about 90 microns, equivalent radius, with the same sample of cement as was used in this work. When the density of the cement is taken as 3.295, the density of the oil as .86 and the viscosity of the oil as 0.18 poises and with a height of suspension of 77.7 cms., the time

corresponding to 90 microns is 5 minutes. This means that the first change in the rate of settling occurred in five minutes and the portion of the fall curve between zero and five minutes was a straight line. If the height of the suspension had been 30 centimeters (the approximate distance in this work) the time would have been approximately 2 minutes. The necessity of a viscous liquid is therefore obvious.

As already pointed out, such a viscous liquid cannot be used in the side tube because of lag, and toluol was used instead. Even with toluol, however, no straight portion of the fall curve was ever obtained. If the powder is finer, say all passing through 325 mesh screen, the situation is much different. Assuming a size of 20 microns equivalent radius for the largest particles and assuming other conditions as already noted, the time for 77.7 centimeters of fall becomes 100 minutes and for 30 centimeters of fall about 37 minutes. A less viscous liquid may then be used, the run shortened in time, no starting troubles met with, and the effect of the lag will be less in the side tube and a less volatile liquid can therefore be used in the side tube.

The results of the mathematical discussion which indicates that there is an error involved in the determination of weight in suspension by the use of the Kelly formula, has not been experimentally confirmed or even investigated in the work. It appears that there will only be a few cases where workers will have subjected themselves to this error since, as pointed out, it is easier to calculate the percent settled values directly from the readings.

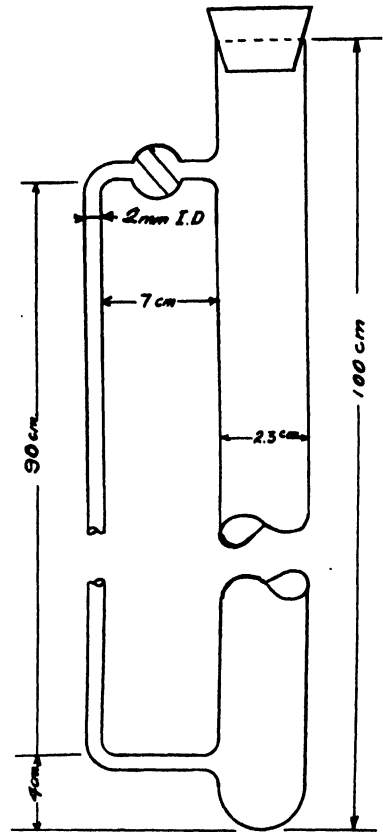


FIG. 5
Modified Form of Wiegner Tube

II. Experimental Work with Modified Wiegner Tube

Apparatus. Since the Kelly tube was not satisfactory for our work and we were loath to abandon the idea or general method, we examined the original apparatus of Wiegner. This tube (with the improvement of Ostwald and von Hahn), was successfully modified by us to provide an apparatus of extreme sensitivity by means of which successful sedimentations of cement were made.

Fig. 5 shows the details of the new glass sedimentation tube. The essential difference between this tube and the tube of Ostwald and von Hahn is that the side tube is bent over and re-enters the main tube at the top so as to provide a connection between the vapor space over the suspension and the vapor

space over the side tube liquid. This connection eliminates differences in vapor pressure due to slight differences in temperature and permits exposure of the vapor spaces to air of the room. Thus a cork, covered with tin foil, may be inserted in the top of the main tube and evaporation prevented.

The tube was suspended from the top by a sturdy clamp and this in turn was supported by a heavy metal pipe resting on concrete supports which were independent of the thermostat tank. The tube was immersed in a constant temperature bath 12" x 12" x 48". The tank contained two windows, 6" wide,

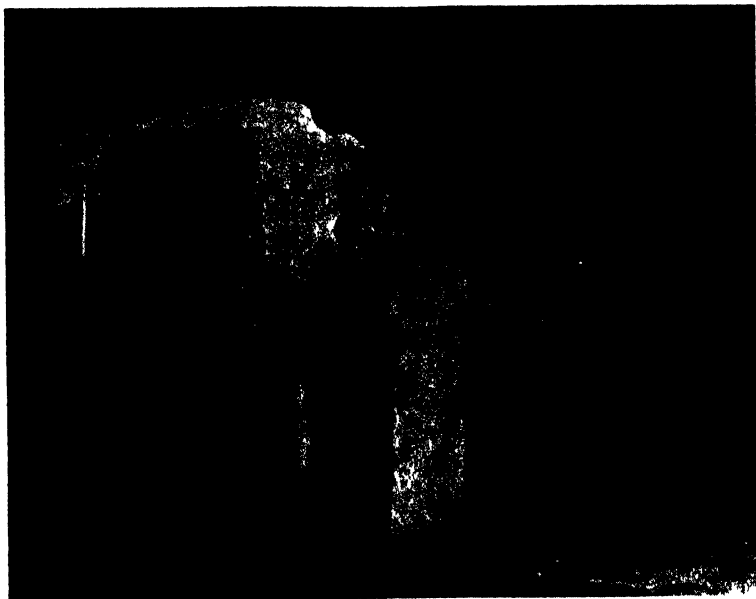


FIG. 6
View of Assembled Apparatus

extending the full height of the tank and placed on opposite sides. The sedimentation tube was placed so that the side tube was close to one window on the inside and a Gertner microscope with micrometer head, mounted on a horizontal support, was placed on the outside of the same window in such a position that the meniscus of the side tube was visible in the field of the microscope. A light was placed on the outside of the opposite window so as to illuminate the meniscus. The assembled apparatus is shown in Fig. 6. Experience showed that the only difficult points about the construction and erection of the apparatus was the elimination of vibrations in the meniscus caused by stirring of the water and also the erection of the tube and microscope on firm supports.

Procedure. The suspensions were prepared in the same manner as previously outlined. Some of the clear liquid used in preparing the suspension was poured into the sedimentation tube and, by tilting, the side tube filled with clear liquid nearly up to the level at which the suspension would stand.

The stop cock was then closed and excess of liquid poured, after which the tube was placed in position. A large funnel was inserted in the top of the main tube and the suspension poured into the tube as rapidly as possible. A stop watch was then started, the cork inserted in the top of the main tube, the clamp of the tube loosened and the tube adjusted so that the meniscus came into the field of the microscope. The clamp was then tightened and readings were taken with respect to time by adjusting the cross hair of the micrometer eyepiece to the meniscus and observing the reading on the micrometer head.

In starting runs with this apparatus, two precautions were necessary. First the large funnel was used in order to prevent wetting the wall of the main tube above the level of the suspension and thereby introducing drainage error. Second, the upper side arm connection which contained the stop cock was kept entirely free of liquid, since if a drop of liquid was present, it found its way to the stop cock and stopped the vapor connection between the side tube and main tube.

A zero reading was obtained by extrapolation backwards. The final reading was taken when there was no change in the position of the meniscus over a period of two days. The microscope used had a scale range of approximately one-fifth of the complete change in level of the meniscus. Therefore, it was necessary to shift the position of the meniscus whenever it passed out of the field of the microscope. This was done by raising the tube. The tube could be shifted and the cross hair again brought to the meniscus within one minute. The last few readings taken before the shift and the first few readings taken after the shift were plotted and the two curves extended, the first one ahead and the second one back. Thus two readings were obtained for the same time value and the difference between the two readings represented the distance through which the tube had been raised, expressed in terms of micrometer head readings. It was only necessary to add this difference to all readings taken after the shift. When the second shift was made, the addition to be made consisted of two such differences.

In order to calculate the observed readings over to percent settled, the readings were tabulated in a continuous series which included the values for the shift of the tube. From these readings the value of the reading for zero time was subtracted which resulted in values of reading drop for the corresponding times. Using a slide rule, percent settled was calculated from reading drop by dividing the reading drop for the time in question by the total reading drop and multiplying the result by 100.

Data and Calculations. A preliminary test was made to determine the amount of evaporation loss. The side tube was filled with clear liquid and the tube adjusted to bring the meniscus into the field of the microscope. The cross hair was adjusted to the meniscus and the apparatus allowed to stand for five days with frequent observation. At all times the cross hair was found to be directly on the meniscus.

In order to determine the amount of the lag in the modified apparatus, the tube was filled with clear oil and the cross hair of the microscope brought to the meniscus. Approximately one-third cc. of oil was then added to the

large tube and the cross hair again brought to the meniscus as quickly as possible. It was found that approximately 200 divisions change were produced and that the position of the meniscus was constant after 20 seconds.

Preliminary observations showed that a change of one-half inch in the level of the water in the thermostat bath produced an observable change in the position of the meniscus.

It was found that there was an uncertainty in locating the position of the cross hair on the meniscus amounting to five divisions in either direction.

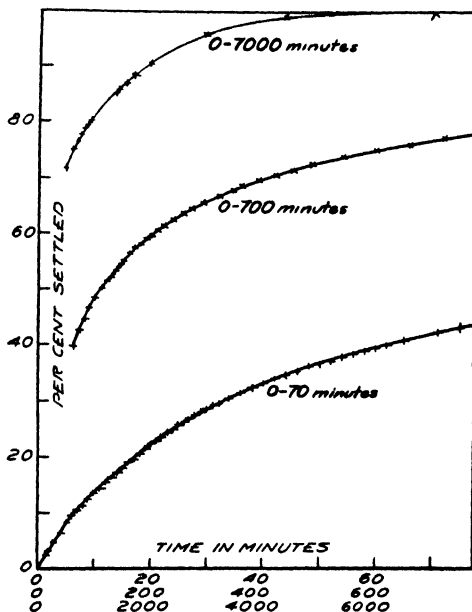


FIG. 7
Fall Curve Cement No. 2

Later work showed that as a rule the entire range through which the meniscus moved amounted to about 7500 divisions. Thus the measuring error was less than 0.1 percent.

It was observed that, when the temperature of the thermostat varied by more than 0.02 C., measured by a Beckmann thermometer, a variation in the position of the meniscus could be observed.

Fifteen sedimentation runs were made with this apparatus and twelve were successfully completed as far as the apparatus was concerned, *i. e.*, some of them were not considered as satisfactory because of lack of peptization or for other reasons not associated with the apparatus. Two runs were unsuccessful because of poor supports and one because of the presence of a drop of liquid in the stop cock of the upper side arm connection.

The data for one run are given in Table IV, and are also shown in curve form in Fig. 7.

TABLE IV

Sedimentation of Cement Sample 2, using newly Modified Tube

Weight of cement taken 3.0 grams. Dispersed in 300 cc. of sedimenting oil (65% stanolind 35% kerosene) by heating for 20 minutes and adding small piece of camphor. Dispersion stood in thermostat overnight with cover over jar. Temperature of thermostat 29° C. Density of cement 3.295. Density of oil (determined after sedimentation was complete) .8602. Viscosity of oil .1813 poises. Height of suspension 77.7 cms.

Reduced form of Stokes equation $r = (.000407/t)^{1/2}$
(for time in minutes)

Time (min.)	Reading	Reading drop	Percent settled
0	-25	000	00.00
1.5	148	173	2.26
2	211	236	3.08
3	331	356	4.65
4	452	477	6.22
5	594	619	8.08
6	688	713	9.29
7	765	790	10.30
8	846	871	11.34
9	931	956	12.47
10	1004	1029	13.40
11	1073	1098	14.31
12	1152	1177	15.34
13	1225	1250	16.30
14	1295	1320	17.20
15	1365	1390	18.11
16	1434	1459	19.00
17	1500	1525	19.87
18	1569	1594	20.80
19	1928	1653	21.55
20	1685	1710	22.30
21	1736	1761	22.97
22	1790	1815	23.65
23	1848	1868	24.35
24	1893	1918	25.00
25	1950	1975	25.75
26	1989	2014	26.22
27	2043	2068	26.97
28	2089	2114	27.55
29	2128	2153	28.05
30	2185	2210	28.85
31	2213	2238	29.20
32	2253	2278	29.70
34	2332	2357	30.72

TABLE IV (Continued)

Time (min.)	Reading	Reading drop	Percent settled
36	2404	2429	31.70
38	2464	2489	32.50
40	2532	2557	33.38
42	2587	2612	34.10
44	2650	2675	34.90
46	2714	2739	35.75
48	2771	2796	36.50
50	2812	2837	37.00
52	2853	2878	37.55
54	2904	2929	38.15
56	2947	2972	38.80
58	2987	3012	39.30
60	3028	3053	39.82
62	3073	3098	40.40
65	3129	3154	41.20
71	3243	3268	42.70
75	3314	3339	43.55
80	3404	3429	44.75
85	3486	3511	45.80
90	3558	3583	46.80
95	3625	3650	47.65
100	3694	3719	48.50
105	3754	3779	49.30
110	3818	3843	50.20
115	3873	3898	50.85
120	3924	3949	51.55
125	3977	4002	52.25
130	4023	4048	52.85
135	4069	4094	53.50
140	4121	4146	54.00
145	4165	4190	54.70
150	4207	4232	55.20
160	4297	4322	56.45
170	4377	4402	57.50
180	4445	4470	58.30
190	4508	4533	59.15
200	4568	4593	59.90
210	4626	4651	60.75
220	4683	4708	61.55
235	4758	4783	62.50
255	4845	4870	63.55
270	4911	4936	64.40
290	4992	5017	65.45
300	5033	5058	66.00

TABLE IV (Continued)

Time (min)	Reading	Reading drop	Percent settled
320	5101	5126	66.90
345	5174	5199	67.80
360	5226	5251	68.60
390	5307	5332	69.60
420	5382	5407	70.60
450	5444	5469	71.40
485	5517	5542	72.75
540	5630	5655	73.90
600	5733	5758	75.02
660	5816	5841	76.25
720	5903	5928	77.40
780	5979	6004	78.90
840	6051	6076	79.30
900	6116	6141	80.10
1335	6493	6518	85.00
1400	6544	6569	85.75
1520	6639	6664	87.00
1680	6750	6775	88.50
1955	6899	6924	90.40
2925	7308	7323	95.50
4350	7552	7577	98.75
7020	7637	7662	100.00

Discussion of Results. The fact that the apparatus stood for five days without any observable change in the meniscus while clear oil was in the apparatus and the tube sealed with the cork, can be interpreted in no other manner except as evidence that no evaporation took place. None of the previous workers except Kelly have made provisions to prevent evaporations and Kelly's provisions have been found to be inadequate in this work.

Since viscous oil was also used in the side tube as well as the main tube, and the lag was not more than 20 seconds for a movement of 200 divisions of the meniscus, we conclude that lag has been practically eliminated and largely by the reduction of movement of the side tube liquid to a very short distance which amounts to about 4 mm. Another advantage is that only a small volume (about 0.0012 cc.) of clear liquid is transferred to the main tube over a period of several days.

The observation that the error in reading was less than one tenth percent, appears to be well borne out by the curve of Fig. 7, where the data falls on a smooth curve with very satisfactory regularity.

The chief reason for the success of the apparatus is in the ease with which the manipulations can be made and especially the ease with which the run can be started. The fact that the suspension need not be poured in to a definite level, but can be dumped in and the tube shifted to obtain the desired level of the meniscus, is a most important factor in starting runs.

The reason that the water level of the bath must be kept reasonably constant in order to prevent effect on the position of the meniscus is probably that a slight bending of the pipe support occurs and thereby elevates or lowers the tube.

The importance of temperature control in this apparatus indicates the sensitivity of the apparatus. Incidentally, it emphasizes a point not so well recognized, i.e., with sedimentations of this type, constant temperature is more important than in some other types of sedimentation.

Conclusions

1. The formula proposed by Kelly for use with his sedimentation apparatus appears to neglect the important factor of leakage to the larger tube, when the formula is used to determine weight in suspension directly. A formula is proposed which is believed to be more accurate.

2. If percent settled is calculated directly from the readings, the constants appearing in Kelly's equation need not be determined and the experimental work is considerably simplified.

3. The sedimentation tube proposed by Kelly is not satisfactory for the sedimentation of cement when the bulb used to protect against evaporation is blown in the upper end of the manometer side tube, as this makes the tube more difficult to handle and increases the possibility of spoiling experimental runs.

4. Kelly's tube or modified form of this tube do not entirely protect against evaporation and are not satisfactory for sedimentation of cement, for this reason.

5. The protection bulbs proposed by Kelly, although objected to on the grounds that they increase manipulative difficulties do reduce evaporation to a considerable extent, although not completely.

6. Kelly's tube or any modification of it where the tube contains a side tube in an essentially horizontal position, and where readings depend on the travel of a long column of liquid over substantial distances, is not satisfactory for the sedimentation of cement samples which contain a large proportion of coarse particles and thereby produce rapid movement of the meniscus in the early stages of settling, even when viscous liquids are used as the suspension medium, leading to lag in the side tube readings and thus masking the rates of change.

7. The use of a viscous liquid as the suspension medium requires that means be taken to prevent wetting of the main tube wall above the level of the suspension, for example, when adding the suspension, for if the wall is wet, the liquid drains slowly down and causes erroneous results in the first few minutes.

8. Kelly's tube has the disadvantage that the level of the suspension must be perfectly definite and as exactly as possible to a predetermined mark, otherwise the meniscus will not come to equilibrium in a position to permit

utilization of the scale. Attempts to realize this level in the short time permitted when working with cement, result in many failures and much loss of time.

9. The force by which the meniscus is brought to the equilibrium position in Kelly's tube is very slight and the meniscus is very easily stopped or arrested. With further refinement, it should be possible to reduce arresting of the meniscus but it is likely that there will always be a portion of experimental work lost from this cause.

10. The modified Wiegner apparatus developed in this work entirely eliminates evaporation loss and is the only apparatus for sedimentation which does this successfully. Kelly's tube, which is the only other sedimentation apparatus attempting to prevent evaporation loss, is inadequate for this purpose. If evaporation is not prevented, results of any work done with sedimentation tubes of the Wiegner type are of doubtful value.

11. The modified apparatus has almost no lag and permits the use of viscous liquids in the side tube, approximately 200 divisions of change being produced in less than 20 seconds with a viscous liquid.

12. The measuring error of the apparatus is less than one tenth percent of the total range covered.

13. The apparatus developed in this work is easy to operate and is relatively free from manipulative hazards, due to the fact that the level of the suspension need not be adjusted to any definite value, but the tube is either raised or lowered as required. This is of special advantage in carrying out sedimentations of cement, where a substantial amount of relatively coarse particles cause rapid movement of the meniscus during the early stages of settling, since there is little difficulty in obtaining a reading at the end of the first minute.

14. When using oil as the suspension medium, as was done in this work, very careful control of the temperature is required. A variation of not more than 0.01 C° may be permitted.

15. Considerable opportunity is afforded with this modified Wiegner apparatus for the investigation of much more dilute suspensions than have been studied. The microscope can be equipped with an objective to give greater magnification and the movement of the meniscus still further reduced.

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COHERENT EXPANDED AEROGELS

BY. S. S. KISTLER

Introduction

There are few topics in colloid chemistry that have experienced such extensive investigation as that of the structure of gels. Numerous hypotheses have been presented and supported by experimental data of one character or another. Certain aspects of gels have seemed to classify them as solid solutions, others as emulsions, while yet others give strong support to a two-phase solid-liquid structure. Although the latter theory in similar form to that postulated by Nägeli in 1858 has been accepted by most of the foremost colloid chemists, the evidence has not been sufficiently unequivocal to convince all.

The evidence presented in the results of diffusion experiments through gels, the fact that the electrical conductivity, refractive index, and vapor pressure before and after setting are identical, at least in certain cases, and the known facts of syneresis would seem to leave little room for doubt of the two phase nature of gels in general. A theory, in order to be perfectly acceptable must, however, enable verifiable predictions to be made from it.

Thomas Graham¹ showed that the water in silica gel could readily be replaced by organic liquids, and Bütschli² demonstrated the same fact for gelatin. Every biologist takes advantage of this discovery in making microscopic sections. In that process the water is successively replaced from the gelatinous tissues by alcohol, xylene and paraffin. The final result is a gel in which paraffin is the disperse phase instead of water. In spite of the commonness of this replacement process and the early date of its discovery, very little of a theoretical nature has been made of it. Theories of the origin of swelling in the elastic jellies very commonly ascribe a high place to osmotic forces, but no explanation is offered for the fact that these same gels retain their swelling, and in fact offer great resistance to compression, when the water is replaced by a liquid such as benzene in which osmotic forces must be negligible owing to the insolubility of the material of the jelly.

Gelatinous membranes such as animal membranes, nitro-cellulose, and cellophane³ have frequently been used as ultrafilters. In many cases there can be no question but that the membrane acts as a sieve. It has been conclusively shown that the water in these membranes can be replaced by other liquids without impairing the sieve action.³ The membranous filters have demonstrated particularly well the resistance to compression offered by swollen gels, both in water and in other liquids. Unswollen cellophane will not permit any liquids to pass, and even gases at high pressure pass in scarcely

¹ J. Chem. Soc., 17, 318 (1864).

² "Über den Bau quellbarer Körper," Gottingen (1896).

³ J. W. McBain and S. S. Kistler: J. Gen. Physiol., 12, 187 (1928).

detectable quantities. When it is swollen by merely wetting in water, however, it passes many liquids with ease. In both aqueous and nonaqueous solutions the author has conducted filtrations at as high as 120 atmospheres pressure with little evidence of compression of the swollen membrane.⁴ An interesting fact is that Rodewald⁵ found that starch swelled against 2,500 atmospheres pressure.

Whatever may be the explanation of swelling, the evidence is indisputable that when many gels are once swollen they show a resistance to recompression that is independent of the liquid within their meshes. When once transferred from water to a nonswelling liquid they show very obvious indifference to what liquid it may be.

In the face of what has been presented, the emulsion theory is completely untenable except, perhaps, in some very special cases, so that no further thought will be given to it.

In spite of the above facts, it can still be argued that the liquid is of large importance in the constitution of the jelly. Many chemists are of the opinion that in the hydrophyle jellies much of the water is held as shells surrounding the colloid particles. If such were the case, the mobility of the water should be of a different order from that of pure water. That hydration phenomena exist can scarcely be disputed, but the author⁶ has definitely demonstrated that the average viscosity of the water within a gelatin jelly and certain of the inorganic jellies is not widely different from that of pure water. The structure of these jellies, when once formed, is very likely only mildly dependent upon solvation.

Such facts as those above, and others, have led me to the conviction that when once formed, a jelly is in general independent of the fluid filling its meshes, and this fluid might just as well be a gas as a liquid. The fact that all coherent jellies are filled with a liquid is accidental and of little significance.

In support of this assumption, one immediately recalls that silica, alumina, and ferric oxide jellies may be dried to a hard glassy mass which is yet porous and undoubtedly retains a vestige of the original structure. Even gelatin jelly that has been transferred to alcohol or benzene shows the same phenomenon.⁷ It might be argued that the relationship here is extremely distant. Still a further bit of evidence comes to the support of the assumption. Bechhold⁸ and others have been in the habit of measuring the pore diameter of a membrane for ultrafiltration by means of the so-called bubble test. The membrane is supported with water above it and air pressure applied below until bubbles begin to form on the upper side. From the known surface tension of the water and the measured pressure, the diameter of the pore can be calculated. With number 600 cellophane, copious bubbling occurs at pressures below 70 at-

⁴ J. W. McBain and S. S. Kistler: *Trans. Faraday Soc.*, **26**, 159 (1929).

⁵ Rodewald: *Z. physik. Chem.*, **24**, 193 (1897).

⁶ S. S. Kistler: *J. Phys. Chem.*, **35**, 815 (1931).

⁷ Bachmann: *Z. anorg. Chem.*, **100**, 1 (1917).

⁸ *Z. physik. Chem.*, **60**, 257 (1907). An error in Bechhold's calculations was corrected by S. L. Bigelow and F. E. Bartell: *J. Am. Chem. Soc.*, **31**, 1197 (1909).

mospheres. If the pressure is released, the passages refill with water and it again requires the same pressure to start bubbling. The membrane is found to have suffered no deterioration by the experiment. Now it was shown earlier that a collapsed membrane of cellophane would not permit any gas to pass, so that the only conclusion to be drawn is that the water has been forced out of the meshes of the gel in a small area, and in that region we have a gel with air as the continuous phase.

If one chooses to produce an aerogel by replacing the liquid in a gel with a gas, it is found that as the liquid is evaporated, the gel collapses until it has been reduced to a mass very small compared with the original gel. The very apparent explanation is that as the surface of the liquid tries to recede within the structure of the gel, the capillary effect combined with the high tensile strength of liquid crushes the gel to the point where the structure is strong enough to withstand the force. With unhardened gelatin that point is not reached until practically all of the liquid is gone, while with silica and similar gels complete collapse does not occur and a porous mass remains. Obviously if one wishes to produce an aerogel, he must replace the liquid with air by some means in which the surface of the liquid is never permitted to recede within the gel.

Experimental Procedure

If a liquid is held under a pressure always greater than the vapor pressure, and the temperature is raised, it will be transformed at the critical temperature into a gas without two phases having been present at any time. Actually under these conditions there is no transformation at the critical temperature. The change that does occur is gradual and continuous over the entire range of temperature, and a small increase in temperature from slightly below the critical temperature to slightly above has no more meaning to a gel in the liquid than a similar change in temperature any other place in the temperature range. Accordingly, it becomes possible to take a gel filled with a liquid, transform the liquid gradually into a gas, allow the gas to expand above the critical temperature, and end with the gel filled with gas of low density without at any time having subjected the gel to compressive forces. This, in general, is the procedure that has been followed in the present investigation.

A small autoclave of 75 cc. capacity, capable of withstanding at least 300 atmospheres pressure, was used. It was heated in an electric furnace.

At first the attempt was made to work with the inorganic jellies filled with water. Both the pressures and temperatures are inconveniently high, but a more serious difficulty was encountered. At temperatures approaching the critical, water becomes such a powerful solvent and peptizer that gels such as silica and alumina were completely peptized. At first it was thought that the peptizing action was due to alkali from the glass vessels containing the gels within the autoclave. Careful elimination of this source of alkali did not prevent the peptization, however. For example, a piece of silica gel would go into solution as the temperature rose and later when the density of the solvent became too low would precipitate as a very voluminous powder. The powder

thus formed was undoubtedly crystalline, although no attempt was made to prove it other than to show that adsorption of water vapor in it was negligibly small as compared with adsorption in normal silica gel.

With the inorganic gels it was found satisfactory to replace the water with 95% ethyl alcohol. Occasionally they were transferred from the alcohol to ethyl ether because of the lower critical temperature and of the smaller chemical activity. The organic gels were always transferred from alcohol to ether, thence to petroleum ether and finally to liquid propane, except in the case of nitrocellulose which, on account of its solubility in alcohol and ether, was formed in benzene and transferred directly to the petroleum ether.

The principal precaution to be taken at this stage is always to replace one liquid with another with which it is completely miscible. If the two liquids are not miscible, an interface forms between the liquid in the gel and that outside and can, under circumstances, compress the gel just as a liquid-gas interface does when the gel is dried. Usually it merely serves to prevent exchange of the liquids.

In the present study only firm jellies were investigated since it seemed probable that they would be more independent of the liquid than the gelatinous precipitates. Also the optically clear jellies lend themselves better to observations on internal changes.

Results

Silica. Silica gels were chosen to experiment with first because of the ease of preparation and the mechanical character of the product. The gels were usually prepared by pouring slowly with stirring a solution of "N" brand water glass of specific gravity 1.15 into an equal volume of 6 N hydrochloric acid. The solution was then filtered and set in paraffined crystallizing dishes to harden. After 24 hours sufficient syneresis had set in to loosen the gels from the dishes and enable them to be removed and placed in wash water. Washing was continued until the water showed no chloride ion.

The biologists' experience is that sudden transference of tissues into alcohol produces large shrinkage and distortion. With this in mind, two samples of gel were chosen from the same batch, one was placed immediately in 95% alcohol and the other was transferred by gradual stages extending over several days. The percentage silica in the two resulting aerogels was identical, showing that sudden transfer is quite satisfactory. None of the inorganic jellies used required gradual transference.

The whole cycle of heating to the critical temperature and liberating the gaseous alcohol usually took about an hour and a half. Probably no significance should be attached to the time involved.

The aerogel obtained is transparent and highly opalescent, and occupies nearly the same volume as the aquogel.

The aquogel produced as above contains about 8% silica, so that allowing for about 20% volumetric shrinkage upon removal of the alcohol, and assuming a density of 2 for the silica, the aerogel consisted of 5% SiO_2 by volume, and the remainder air. The structure is so fine, however, that the aerogel is

quite transparent, transmitting light of a slightly orange tint due to the very pronounced scattering that occurs on account of the inhomogeneity of structure. The scattered light is bluish, as would be expected. The aerogel shows considerable resistance to crushing, considering its low percentage of silica, and is quite resilient. When a small piece is dropped, it emits a metallic ring and bounces without fracture.

This material should prove excellent for ultramicroscopic study of gel structure on account of the very large difference in refractive index between phases. An examination of a piece with 1700 diameters magnification and horizontal illumination showed nothing but uniform light due to the Tyndall Effect. Even with thin fragments obtained by fracturing a piece of the silica aerogel the thickness was so large that it was likely that the uniform Tyndall light masked fine structure that might otherwise have been visible. Accordingly, silica gel was cast under a cover glass on a microscope slide and converted into aerogel in the usual manner. Numerous points of light were visible with transverse illumination, but nothing could be seen of what might be called a structure.

Substage transverse illumination proved impractical on account of the small refractive index of the aerogel. The light is completely reflected at the surface of the slide and does not penetrate into the gel. Perhaps some interesting observations could be made with the aid of the new Spierer lens. My cursory microscopic examination proved too meager for any conclusions.

Since silica aerogels are so easy to obtain, a more detailed study was made of them than of any of the other aerogels produced. It was found possible to produce silica aerogels with any percentage silica by volume from 2% up to the dense product of commerce without special precautions, and I feel certain that 2% is not the lowest obtainable concentration. For gels above 5% silica, the usual procedure was to precipitate a gel as described above and then dry it slowly until the desired concentration was reached. Such a dried gel shows very decided evidence of the compression to which it is being subjected. Upon placing it either in water or in alcohol it reswells to a certain extent dependent upon the concentration of the silica, and perhaps upon details of past history. The elasticity of the silica fibrils is very well shown by this effect. The second column of Table I shows the increase in volume upon placing pieces of gel in

TABLE I

% SiO ₂ by wt.	Percentage change in volume	
	upon placing in alcohol	upon removal of alcohol
8.3	—	—28.1
8.3	+ 2.7	—12.0
9.7	+ 3.7	—22.5
13.6	+ 4.7	— 1.2
17.8	+ 9.6	— 0.7
20.7	+12.2	— 0.9
23.7	+ 9.7	— 1.7

95% alcohol after drying them from an original concentration of 8.3% by weight to the weight percent shown in the first column. Volumetric changes were computed from measurements of linear changes.

Column 3 of the table shows the changes in volume upon conversion into aerogels. In each case the temperature at which the alcohol was released from the autoclave was approximately 20° above the critical temperature.

Initial measurements on the gels were made up to a concentration of 70%, but unfortunately the higher members of the series were accidentally destroyed. It seems reasonable to assume that the increase in volume upon placing in alcohol will decline with the higher percentages of silica.

Unless the autoclave is evacuated after release of the alcohol vapor and while still hot, considerable quantities of alcohol remain within and are adsorbed by the gel upon cooling. It is highly possible that the capillary effect of this alcohol is responsible for part at least of the shrinkage observed. That this alcohol is held as capillary condensed liquid seems certain from the fact that if the aerogel is heated to 300° or so for a short time in the air it becomes very much more transparent. If the gel is then allowed to stand exposed to the air at room temperature for a few hours, the opacity increases markedly. Transparency is again recovered upon heating. Undoubtedly here and there through the gel small capillaries become partially filled with liquid and offer discontinuities of a larger order, thus increasing very much the scattering of transmitted light. Increase in intensity of the scattered light is very marked when water vapor amounting to only two or three percent of the weight of the silica has been adsorbed.

From 3 to 4 percent of the weight of the aerogel dried at 350° is water that is removed only at a much higher temperature. Probably this water is adsorbed on the surface of the fibrils as a monomolecular layer. However, I have no reason for believing that none of it is held in chemical combination.

The aerogel can be heated to 700° for considerable periods without change in appearance. When the temperature goes to 900°, some internal change takes place that greatly increases the opacity of the product. Although some shrinkage occurs upon such ignition, the aerogel is still of low density and capable of adsorbing much water vapor. Table 2 gives increase in weight with time, of two samples of gel placed in saturated water vapor. Sample 1 was ignited at approximately 800°, while sample 2 was unheated.

TABLE II

Time	Gain in weight	
	Sample 1	Sample 2
3 days	57%	50%
14 days	157	120
23 days	194	140
placed in water	327	166
Total decrease in vol.	67	81

It is very evident that igniting the gel hardened its structure so that it was very much better able to withstand the compressive forces exerted on it as the capillaries filled with water. The final volume of a gram of sample 1 was 73% larger than the volume of an equal weight of sample 2.

Since surface tension is the principal force tending to collapse a gel, the experiment was tried of heating silica gel in the autoclave to a temperature below the critical temperature and releasing the alcohol. The autoclave was heated to 215°, 28° below the critical temperature. Here the surface tension is small but not negligible, being approximately 12% of the tension at 20°. The result was that a good grade of aerogel was obtained, but it suffered a decrease of 52% in volume and was considerably cracked.

Since the structure of the aerogels is submicroscopic, it is to be expected that they can be ground to an exceedingly fine powder. A piece crushed between the fingers has an unctuous feeling like talc or graphite, rather than the gritty feeling that one expects from silica. Ground in a mortar, the powder occupies as large a volume as the original sample, showing that the structure has been very little damaged. Both microscopic examination of the powder and sedimentation measurements indicate many particles of from 1 to 4 microns diameter. Even those of 1 micron diameter must possess a gel structure and be capable of very much finer subdivision.

Alumina. Considerable difficulty was experienced in producing good transparent alumina jellies of sufficient concentration to possess the mechanical strength necessary to assure ease of handling. The method finally adopted was to precipitate the jelly from a colloidal solution of aluminum oxide made by the dialysis of a solution of aluminum acetate (Gann's method). If dialysis was carried far enough, a very small amount of sulfate ion was sufficient to cause a 1% sol to gel. After gelation, the gel was allowed to dry down to the desired concentration. It was found impractical to wash out the small quantity of sulfate on account of the peptizing action of pure water. The attempt was made to wash the gel in dilute alcohol solution in order to prevent peptization. The lump of gel swelled, showing distinct evidence of laminations running parallel to the surface of the test-tube in which it was cast. It then disintegrated into elongated transparent platelets whose lengths were several times their breadths. With a little agitation, the whole mass was completely peptized.

When the desired concentration was reached, the gel was transferred to 95% alcohol and after suitable time had elapsed for the exchange of the water and the alcohol, the alcohol was removed in the autoclave.

The lowest density aerogel so far produced was one of alumina in which the apparent density measured 0.02 grams per cubic centimeter. If one accepts the value for the density of amorphous aluminum oxide as 2.6, that means that in one cubic centimeter of transparent aerogel there was but 0.008 cc. of solid. Needless to say that the gel was exceedingly fragile.

Since with the dilute gels density determinations by means of the displacement of mercury were impractical on account of the danger of crushing the gel, densities were regularly determined by measurement of the displace-

ment of hour-glass sand. The method does not yield highly accurate results, but for rough measurements it is quite practical.

Tungstic Oxide. The attempt was made to produce an aerogel of tungstic oxide. The gel was precipitated from 12% sodium tungstate by means of 6N HNO_3 . It was a light yellow opaque gel with very little mechanical strength. It was cast in short sections of wide glass tubing in order to support it during the processes of washing in water, transference to alcohol, and removal of the alcohol in the autoclave. At the critical temperature, alcohol was found to reduce it badly, so that it was found advisable to transfer from the alcohol to ether before placing in the autoclave.

The final product was bluish in color. It occupied the same volume that the aquogel had occupied and seemed to have lost little of what strength it had originally. The forces between the micelles seem particularly weak. It is highly possible that with a study of the conditions of formation, a very much stronger gel could be obtained.

Ferric Oxide. A ferric oxide sol was made by adding ammonium carbonate to ferric chloride, dialyzing for four weeks and then concentrating at a low temperature on the water bath to a syrupy liquid analyzing 8.8% Fe_2O_3 . To a part of this sol a dilute solution of potassium sulfate was added a drop at a time with violent shaking between additions until upon standing for half an hour a firm vibrating jelly formed. The remainder of the sol was placed in a test tube and heated in a beaker of boiling water. In the course of half an hour it had set to a firm jelly resembling in every way the one precipitated with sulfate. These jellies were clear and transparent in thin sections. Both were placed immediately in 95% alcohol.

Several attempts to obtain a clear aerogel directly from alcohol met with complete failure. The product each time was a red, opaque, pulverulent mass. Transferring the gel from alcohol to ether and removal of the ether in the autoclave proved equally unsuccessful. Finally by transferring to ether, then to petroleum ether and finally to propane and removing the propane in the autoclave, a good grade of aerogel was obtained. The pieces had small mechanical strength, were very dark red and transparent in thin sections. The density measured 0.2.

At first it was thought that the gel structure must be composed of a hydrate, and the failures in alcohol and ether could be attributed to dehydration at the higher critical temperatures. It was found, however, that the aerogel formed in propane could be heated to 400°-500° without any evident change in structure so that the hydrate hypothesis became untenable. Perhaps the explanation will be found in chemical changes due to the alcohol or water. Even in the ether there were undoubtedly traces of both water and alcohol.

Stannic Oxide. A sol of stannic oxide was made by the hydrolysis of stannic chloride and the peptization of the precipitate with ammonia. Upon slow evaporation, the sol set to a firm jelly. This jelly was allowed to concentrate by evaporation. It was then washed free of most of its water in 95% alcohol, transferred to ether, and the ether removed in the autoclave. The

aerogel formed was slightly tinged with yellow, opalescent and beautifully transparent. Its density was not measured but was estimated to be well below 0.1. Heating to 400° for two hours caused no visible change.

Nickel Tartrate. Nickel tartrate was dissolved in ammonium hydroxide solution and set in an open crystallizing dish to permit the ammonia to evaporate. By slight variations, it was found possible to produce gels that analyzed only 0.14% NiO. The gel from which the aerogel was made analyzed 2.09% NiO. It was demonstrated, however, that the gel substance was actually nickel tartrate so that the gel contained 5.8% solid. It was light green, firm and quite transparent.

Samples were converted into aerogels from both alcohol and ether. That from the latter liquid was the most transparent. The aerogel from both solvents was very fragile. Parts were opaque but many fragments had good transparency, showing that the submicroscopic structure persisted after removal of the liquid.

Cellulose. In spite of the complete success with the inorganic jellies, it remained doubtful if the elastic jellies would yield aerogels. Having studied the properties of cellophane a good deal and having been impressed with the resistance that it offers when swollen to compression either in aqueous or non-aqueous solutions, I felt more sure of success with it than with gelatin. Preliminary experiments trying to obtain swollen cellophane as an aerogel from ether failed. Failure might well have been due to the high critical temperature of ether, 193.8°. Cellulose rapidly undergoes chemical changes at that temperature.

It was thought that perhaps a small amount of residual moisture might have an effect, so some swollen cellophane was extracted with ether in a Soxhlet extractor for several hours, keeping a copious amount of fresh, fused CaCl_2 in the distilling flask. The ether was then replaced by propane and the propane removed at 115°. The cellophane was found to have retained its swelling. It appeared white to reflected but translucent to transmitted light. Upon wetting, it became transparent, and during subsequent drying it shrank to its original thickness and had the appearance of ordinary cellophane.

A solution of viscose was made by xanthating cotton. It was filtered and allowed to stand in the laboratory until it gelled (about a week). The jelly was sliced, washed, transferred to propane in the same manner as the cellophane and the propane removed in the autoclave. The product was dense white and was translucent only in thin layers. Its mechanical strength was poor. The probabilities are that if the jelly had been precipitated with electrolyte after the manner of the formation of cellophane or rayon, it would have possessed much more strength as an aerogel. A piece of this cellulose aerogel was set in a watch glass with enough oil to surround it but not to cover it completely. In the course of a short time the oil had displaced the air completely, leaving a transparent jelly of cellulose and oil.

Nitrocellulose. Enough collodion (du Pont's Parlodion) was dissolved in a 50-50 mixture of alcohol and ether to make a very viscous sol. This was poured into a crystallizing dish, a piece of filter paper laid on the surface of the

sol to prevent convection currents, and covered with benzene. In the course of a few days the entire mass of collodion sol had set to a firm jelly. This was sliced, washed in benzene for several days, then in petroleum ether and finally transferred to propane. The propane was changed several times before the jelly and propane were placed in the autoclave.

The aerogel was very light and tough. It had suffered no shrinkage in the autoclave as far as could be told, but was far stronger than the jelly in benzene. It was translucent even in pieces several millimeters thick. It offered large resistance to compression, but when once compressed it did not reswell to its original thickness. This is the strongest and toughest aerogel that has so far been produced.

Gelatin. When it was attempted to transfer a 5% gelatin jelly to alcohol, it was found practically impossible to make the transfer without large shrinkage and the gelatin's turning opaque. By hardening the jelly first with formaldehyde and transferring to alcohol containing formaldehyde by gradual stages, it was possible to replace the water with alcohol without much shrinkage, and the alcogel was semitransparent. It was much easier to obtain a good alcogel starting with 20% gelatin jelly.

Transference was made to propane in the same manner as with cellulose. It was found particularly necessary to reflux in dry ether to remove the small residue of moisture, since otherwise at the temperature reached in the autoclave (105°) the gelatin would become a viscous liquid and when the propane was released it would swell up like a marshmallow.

The aerogel from 20% gelatin showed no signs of having shrunk in the autoclave. It was white, strong, hard and brittle, and was completely opaque except along thin edges. That from the 5% jelly hardened with formaldehyde was dead white and resembled strong pith in its physical characteristics.

Agar. A 4% agar jelly can be transferred immediately to 95% alcohol without evident shrinkage. The jelly in water is translucent, and retains the same appearance in alcohol. The aerogel was very readily obtained after the manner of cellulose and gelatin. It was dead white and had the characteristics of soft pith.

Egg Albumin. An egg was hard boiled and the white converted to an aerogel in the same manner as above. The aerogel was dead white, hard and brittle. It had relatively little strength and could be fairly readily crumbled in the fingers.

Rubber. Swollen rubber is a jelly of a very different nature from those described above. It would be very interesting indeed if it could be shown that rubber could be converted to an aerogel in the same manner as the aquogels. Immediately, difficulties were met that place rubber in a class by itself. The first step necessary in the formation of an aerogel is the replacement of the swelling solvent by an inactive solvent with a sufficiently low critical temperature. It was immediately found, and the experience is not new, that as soon as it is attempted to replace the swelling solvent with a non-swelling liquid, the rubber shrinks down to its original volume.

My observations would favor the theory that rubber is a two-phase system, one phase being a network or sponge of crystalline fibers and the other being a very viscous liquid held within the network. A swelling liquid would then dissolve in the liquid phase and distend the rubber. The attempt to replace the swelling by a non-swelling liquid would result merely in washing the swelling liquid out of the rubber, rubber and the non-swelling liquid being immiscible.

This theory would predict that if one should swell rubber in such a liquid as ether and then cool to a sufficiently low temperature, the liquid phase of the rubber might be precipitated out on the crystalline skeleton and leave an open gel structure similar to that found in the aquogels. It would then be possible to replace the ether with a non-swelling liquid, e. g., alcohol.

This experiment was tried, cooling the ether gel to the temperature of solid carbon dioxide. As was expected, it now became possible to replace the ether with alcohol, and upon warming to room temperature the alcogel of rubber persisted. In the course of a day or two the rubber had driven out the alcohol and consequently had shrunk to something like its original proportions.

Unvulcanized rubber was used in this experiment. The probabilities are that vulcanization would increase the permanence of the alcogel.

An attempt was made to produce an aerogel by swelling rubber, transferring to liquid carbon dioxide and removing the CO_2 above its critical temperature. This undertaking ended in failure, as was expected. The CO_2 dissolves in the rubber, and when the pressure is released instead of diffusing out through the meshes of a gel structure it must diffuse through the viscous liquid phase. The consequence was that many gas bubbles were formed within the rubber, and these decreased in size only slowly.

I am of the opinion that a good rubber aerogel can be made by swelling vulcanized rubber, cooling it to the point where good replacement of the swelling by a non-swelling liquid can be effected, eventually filling the structure with such a substance as liquid nitrogen, that has a critical temperature so low that the rubber is still rigid, and allowing the nitrogen to escape above its critical temperature. The surface tension of nitrogen is so low that the rigid rubber gel might not be much compressed if the nitrogen were merely allowed to boil off.

One possibility remains untried. Rubber gels formed by vulcanizing rubber solution are reported to synerize. If such is the case, it is very likely that the vulcanization has produced the open sponge structure and that with these gels replacement of the solvent can be effected without recourse to low temperatures.

A remark should be made on the effect of rewetting and drying aerogels. In the case of each organic gel, water drew itself through in a few minutes, and the wet gel was then more or less transparent. On drying, the gel shrank to a small horny mass. Directly wetting the inorganic aerogels was usually disastrous, the gel being crushed. On the other hand, if the aerogel was left in saturated vapor until it had had time to partially fill with water, it could then be placed in liquid water with no harm. Subsequent drying caused large

shrinkage, but usually not to the point to which the original aquogel would have shrunk had it been dried. The greater strength of the gel after it has been converted to an aerogel is doubtless due to dehydration of the fibrils.

Discussion and Conclusions

From the number and variety of gels produced, it is evident that the ability to form an aerogel is a general property of gels. It seems that if there are cases in which it proves impossible to convert a normal gel into an aerogel, these cases will be the exceptions.

The formation of aerogels offers a new means of studying the structure of gels. Diffusion experiments can now be performed with gases instead of with liquids, with the attendant great simplification. In the cases such as agar and gelatin in which the aerogel is not transparent, it is evident that the discontinuities are of a size comparable with the wavelength of light, and therefore a microscopic or ultramicroscopic study becomes possible, whereas in the aquogel in which the refractive indices are so similar, structures may be completely invisible. The removal of the solvent now makes it possible to study the gel structures by means of X-rays without the interference of the solvent molecules, which in many cases scatter the radiation as intensely as the substance of the gel.

The nature of the forces between the micelles probably varies from one gel to another, but it does not seem out of place to speculate on the forces in certain cases. Reasoning from the large strength of silica gels and from the small size of the molecule of silicon dioxide, the conclusion seems necessary that the entire structure is knit together by means of primary valency bonds. Secondary bonds would seem entirely too weak to account for the facts. Very likely the forces that hold together the aerogels made by the coagulation of smokes, are secondary. The silica gel and the smoke gel have strengths of vastly different orders of magnitude. The assumption of primary bonds between silica micelles would connote a crystalline structure to the micelles, a conclusion at which Scherrer⁹ arrived from X-ray studies.

On the other hand, it seems more difficult to visualize the existence of primary bonds between molecules of cellulose and its derivatives, but here the length of the molecule and corresponding length of the micelle is sufficient to enable the micelles to interlace to a sufficient extent to give the mass large strength in spite of the weak nature of the secondary bonds. There are two difficulties to this explanation, however, that must be met. When such a substance as cellophane is swollen to twice its original volume and converted into an aerogel, the residual forces between the micelles must be very much reduced along a considerable portion of the length of each micelle. They could retain their original value only where the micelles cross or otherwise come into direct contact. It would seem that the tensile strength of such an aerogel would be very much smaller than that of normal cellophane, while qualitative observations place it in the same order of magnitude.

The second objection to the existence of only secondary bonds between the micelles in cellulose and its derivatives is that if residual molecular forces

⁹ Nachr. Ges. Wiss., Göttingen (1918).

between the micelles could account for the large strength, they would be of such magnitude that upon the removal of the swelling liquid the micelles would be so strongly attracted to each other that they would immediately come together, and it would be impossible to obtain a gel of the original volume filled with benzene or other non-polar liquid, let alone a gel filled with air.

It is plausible that there are spots where the micelles are bound very strongly together and that between these spots there are lengths on each micelle only weakly affected by neighboring micelles. Along these lengths, molecules that are strongly adsorbed by the cellulose can crowd in between the micelles forcing them apart, or in other words swelling the cellulose. Even in the swollen cellulose these spots of attachment will remain attached. Friction between the micelles would account for the resistance to collapse offered by the aerogel.

Such arrangement could explain the brittleness of very dry cellulose and the softening effect of anything contained between the micelles. In the manufacture of regenerated cellulose, lubrication between the micelles becomes important.

A type of gel that deserves consideration here is the thixotropic gel, i.e., the gel that is reversible with mechanical agitation. Two of the gels used, the alumina and the ferric oxide gel, were when first formed thixotropic. The subsequent concentration of the alumina gel destroyed its thixotropic nature, but one can assume that the submicrostructure was very little changed by this concentration. That these two gels were converted to aerogels disproves the hypothesis that they owe their existence to the "ionic cloud" surrounding each micelle¹⁰ as also does it disprove the general validity of the observations of Hauser¹¹ that during gelatin of a thixotropic gel the particles do not actually touch each other. If that really is the case in the bentonite sols studied, it certainly is not so with alumina and ferric oxide.

In conclusion I should like to express my gratitude to Mr. Charles H. Learned for long hours of patient labor in the laboratory, and also to Dr. J. W. McBain for the loan of apparatus and for kindly assistance and advice.

Summary

It was predicted from general considerations and demonstrated experimentally that in general after a gel is formed the liquid phase is accidental and unnecessary.

Aerogels of silica, alumina, tungstic oxide, ferric oxide, stannic oxide, nickel tartrate, cellulose, nitrocellulose, gelatin, agar and egg albumin were made by removal of the water from the normal gels. Rubber offered difficulties not yet surmounted, but the way has been indicated.

The preparation and properties of these aerogels have been briefly described, and some discussion of structure has been included.

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¹⁰ F. Haber. *J. Franklin Inst.*, **199**, 437 (1925).

¹¹ *Kolloid-Z.*, **48**, 57 (1929).

A COMPARISON OF METHODS FOR THE DETERMINATION OF THE AREA OF ADSORBED MOLECULES IN INTERFACIAL FILMS¹

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Introduction

The problem of determining the dimensions of an individual molecule is one that has received considerable attention. There are in general two widely different methods available for attacking this problem. One method is that employed for calculating the dimensions of the molecule from the x-ray diffraction pattern of crystals. This has heretofore been limited in application to solids possessing a crystalline structure. Recently, however, Stewart³ by means of refined methods of measurement, has applied this method to liquids. The other principal method of attack is from the standpoint of surface chemistry. The thickness of films may be determined and calculations may be made from molecular volume data for the area of the molecule.

Surface films are of two types, namely: those formed by insoluble highly adsorbed substances and those formed by soluble and less strongly adsorbed substances. The former may be investigated by measurement of so-called surface pressures and the latter by measurement of surface or interfacial tension. Calculations of molecular area by this latter method have been accomplished (a) by the limiting slope method of Langmuir⁴ or by the similar maximum adsorption method as used by Harkins and Wampler⁵ and (b) by application of the mixture law method of Mathews and Stamm.⁶

In the present research interfacial tension measurements of water against binary organic liquid systems were made. Calculations of molecular area were carried out by, (a) maximum adsorption method, (b) the mixture law method, and (c) a modified mixture law method.

Methods for Calculation of Molecular Area

The methods above mentioned for the determination of the areas of molecules in soluble films entail the determination of interfacial tension values with which, by means of the Gibbs formulation, $q = - \frac{\tau}{RT} \frac{dS_{22}}{d \ln c}$, the areas can be

¹ This work represents part of a program carried out at the University of Michigan under a grant from the Chemical Foundation. Condensed from a thesis presented by Guilford L. Mack to the Graduate School of the University of Michigan in partial fulfillment of the requirements for the degree of Doctor of Philosophy; June 1931.

² Holder of Chemical Foundation Fellowship.

³ Chem. Rev., 6, 483 (1929).

⁴ J. Am. Chem. Soc., 39, 1848 (1917).

⁵ J. Am. Chem. Soc., 53, 850 (1931).

⁶ J. Am. Chem. Soc., 46, 1071, 2880 (1924).

calculated. In this formulation q represents the mols adsorbed per square cm., and S_{23} the interfacial tension in dynes per cm; the other symbols have their usual significance. Mathews and Stamm have obtained interfacial tension data and have made use of both the limiting slope method and the mixture law method. The agreement of the values thus calculated was only fair.

Maximum Adsorption. The maximum adsorption method is based upon the principle first pointed out by Langmuir, that when the slope of the S_{23} , $\ln c$ curve becomes constant, q (the amount of solute adsorbed) is at its maximum value and is independent of concentration. This maximum value of q , which we shall represent as q_L is supposed to represent the number of molecules necessary to form a completely saturated surface layer of adsorbed molecules. Since q_L is expressed in mols/cm², $1/Nq_L$ (N = Avogadro number) gives the number of square cm. per molecule, or the effective area occupied by the individual molecule.

Mixture Law Method. The mixture law as related to surface tension has generally been stated, in effect, as follows: the surface tension of a binary solution is a linear function of the concentration. Mathews and Stamm considered that the law would be equally applicable to the similar interfacial tension relationships and assumed further that the interfacial tension value of a binary solution against water is a linear function of the concentration in the interfacial layer.

The mixture law method of Mathews and Stamm involves the use of the interfacial tension-concentration graph and is dependent on the validity of the mixture law. If on this graph a straight line is drawn between the interfacial tension values of the two pure constituents, this line is assumed to represent the concentration of the interfacial film of the mixtures at the corresponding interfacial tension values. The horizontal distance between a point on this line and the corresponding point on the determined interfacial tension-concentration curve is assumed to give a measure of the excess concentration at the interface. This gives, then, the excess surface concentration in mols/cm². From the Gibbs adsorption equation, the number of mols adsorbed per square centimeter of surface may be calculated. Dividing the latter number by the former gives the thickness of the surface film in centimeters. The dimensions of this equation are:

$$\frac{\text{mols} \times \text{cm}^{-2}}{\text{mols} \times \text{cm}^{-3}} = \text{cm.}$$

If the film consists of a monomolecular layer of oriented molecules, this gives directly the length of the individual molecule. Its area is calculated by means of the equation $a = M/Ndt$, in which d represents density, and t , the thickness of the film in cm. M and N have the customary significance. In carrying out the calculation it is necessary to make the further assumption that it is justifiable to apply density measurements, which are properly a function of the liquid in bulk, to discrete particles of molecular dimensions.

It should be pointed out that Langmuir had previously employed for the calculation of molecular area a special case of this method which was later

developed more generally by Mathews and Stamm. The equation for calculating the thickness of the surface layer was first given by Langmuir in the form:

$$t = q/(c' - c)$$

In this equation c' represents concentration in moles per liter at the surface, and c the concentration in moles per liter in the bulk of the solution. The surface tension values of water and ethyl alcohol are raised by the addition of soluble salts, hence these solutes must be negatively adsorbed. In other words the solvent is preferentially adsorbed, and hence, it follows that the surface layer consists of molecules of pure solvent, and the concentration of solute in the surface, c' , becomes zero. This condition then leads logically to a method for determining the area of the *solvent molecules*. In contrast to this the method applied by Mathews and Stamm gives the area of the *solute molecules*, since in their work the solute is preferentially adsorbed. Their contribution consists in the use of the mixture law to evaluate the factor c' in the above equation.

The values obtained by the so-called mixture law method are obviously dependent upon the validity of this law, while the values obtained by the maximum adsorption method are independent of the mixture law. Attention should be called to the fact that the validity of the mixture law has never been logically established, and further, it is a question whether the fractional constituents of a given mixture shall be expressed as volume fractions, as mol fractions, or as weight fractions. Mathews and Stamm justify their adoption of the mixture law on the ground that the same linear relationship has been reported by Worley¹ to hold for the similar phenomena of surface tension. They state that "for constituents of like polarity against air, which have no orienting effect, the relationship was fairly well followed, as would be expected when there was no concentrating in the interface."

Since Worley reported but one case in which the mixture law appeared to hold, general conclusions based upon his results seem questionable.

The Validity of the Mixture Law. The original formulation of the mixture law was proposed by Rodenbeck.² Other investigators have attempted to discover a relationship between surface tension and concentration of mixed liquids and the formulations proposed by them vary from the original only in the units used for expressing the concentration of the constituents. Much confusion appears to have existed on this point. Some authors have used mol fraction, others volume fraction and still others weight fraction. None of these authors has really justified the selection of units used by him. A critical and comprehensive review of the literature is given by Morgan and Griggs.³ They calculated the effect of employing the different units for a large number of systems, and found that the use of weight fraction units gave least deviation from the linear relationship.

¹ J. Chem. Soc., 105, 267 (1914).

² Inaug. Diss., Born (1879).

³ J. Am. Chem. Soc., 39, 2261 (1917).

They used bulk concentration values in their calculations. Since surface tension is primarily a function of surface concentration the use of bulk concentration values would not be justified in establishing a relationship between concentration and surface tension.

Application of the theoretical considerations of Gibbs¹ would show that bulk concentration and surface concentration must in fact be quite different, so that the mixture law as expressed by Morgan and Griggs would not be expected to hold. In conformity with the second law of thermodynamics it must follow that from a binary liquid system, the constituent of lowest surface tension must concentrate at the interface in order that the free surface energy will be at the lowest level possible. Differences in the dielectric moments of the molecules and differences in the internal pressures of the liquids may cut down the adsorption in certain instances, but in any case the relative molecular concentrations in the interface cannot be the same as the relative concentrations in the bulk of the solution. It is not to be expected, then, that the interfacial tension or surface tension should bear any simple relationship to the concentration in the bulk of the solution. The principal objection to the mixture law formulations, as applied to surface tension, is, therefore, that they express the interfacial tension as a function of the bulk concentration and not as a function of the interfacial concentration.

The surface tension relationships calculated by Morgan and Griggs on the basis of weight fraction were interpreted as indicating a fair agreement with the mixture law. In those cases in which one component of the binary system shows a tendency toward strong preferential adsorption a close agreement would not be expected. Those units which will, when plotted, give a marked sagging of the surface tension-concentration curve are likely more nearly correct than those which show an apparent better agreement.

Methods of Testing the Validity of the Mixture Law. A direct test of the validity of the mixture law as applied to interfacial tension relationships presents a problem of great difficulty since no method is available for the accurate measurement of the interfacial concentration. An approximate method for testing this law might be one based upon the fact that of the two methods available for calculating molecular area, one, the limiting slope method, is independent of the validity of the mixture law, while the other method is directly dependent upon it. The values of the molecular area calculated according to the Langmuir limiting slope method may, by the proper selection of components, be used as a basis for comparison of the values found from the other (thickness of film or mixture law) method. By using the different concentration units for expressing the concentration factor a comparison of the values thus obtained with those obtained by the limiting slope method should serve as a means of testing the validity of the mixture law. This should also provide an experimental basis for choosing the correct concentration units.

As has been mentioned above, if the interfacial tension of a binary mixture of liquids is plotted against the concentration of one constituent in the solution, a curve is obtained which usually sags below the straight line drawn be-

¹ "Scientific Papers," 1, 219 (1906).

tween the interfacial tension values of the two pure constituents. Only a very few cases have been reported in which the interfacial tensions of the mixtures rose above the straight line, and in each of these cases association, ionisation, or other intramolecular changes not characteristic of ideal solutions are known to occur. If, in a nearly ideal solution, the interfacial tension of the mixture is greater than the value calculated from the mixture law, it would seem likely that the wrong concentration units have been used. Now if a system is selected whose constituents have similar polarities and interfacial tensions, the adsorption will be small and only slight deviations below the straight line function will be observed.

If the constituents are chosen so that the density and molecular weight ratios are widely different, use of the correct concentration units should give values conforming to the calculated interfacial tension values. If the wrong concentration units are used interfacial tension values greater than the calculated will be obtained and these units will thus be eliminated from further consideration.

Modified Mixture Law Method. It has been shown that the thickness of the surface film, t , may be determined by dividing the excess surface concentration in mols per cm^2 by the excess surface concentration in mols per cm^3 . Similarly if the total surface concentration in mols per cm^2 were divided by the total surface concentration in mols per cm^3 , this would give another method for determining the thickness of the surface film. The total interfacial concentration in mols per cm^3 is obtained directly from the mixture law and is represented, in the formulation previously discussed, by the symbol c' . The total interfacial concentration in mols per cm^2 is found from q , the increase in concentration due to adsorption, and from the concentration in the surface before adsorption occurs. This latter factor may be assumed to be equal to the $2/3$ power of the concentration in the bulk of the solution. The equation for calculating the thickness of the film may be formulated as follows:

$$t = \frac{q + c^{2/3}/N^{1/3}}{c'}$$

Where c = bulk concentration in mols/ cm^3

and c' = interfacial concentration in mols/ cm^3 .

This method of calculation has been designated as the modified mixture law method. A full explanation of this formulation will be given later.

Method for Measurement of Interfacial Tension. The methods available for the determination of interfacial tension have not been so thoroughly tested with liquid-liquid systems as they have for the corresponding liquid-air systems. The validity of the different equations used has not, in all cases, been firmly established. It is therefore not surprising that much of the interfacial tension data found in the literature is considerably in error. If adsorption at the interface occurs, only static methods are applicable. Of these methods, the sessile drop and ring methods are not sufficiently accurate for exact work. The maximum bubble pressure method has not as yet been extensively applied to interfacial tension measurements. There remain, then,

the methods involving the measurement of the drop volume or the capillary rise. The ease of operation, together with the simple and firmly established formulation for calculating the interfacial tension, led to the adoption of the capillary rise method. It was realized, however, that much more care in cleaning the apparatus and in purifying the liquids was necessary in the capillary method in order to obtain results comparable in accuracy with those obtainable with the drop volume method. On the other hand, the density of the organic liquid used need not be known so accurately as in the drop volume method. This is an important factor to consider in dealing with mixed liquids of widely differing vapor pressures.

The method of Bartell and Miller¹ was selected for use in this investigation.

During the progress of this research, it was necessary to determine the interfacial tension of water against several different liquids which were expensive and which were difficult to obtain in large quantities in the pure state. It became highly desirable to develop a method which would require but a small amount of organic liquid for each measurement.

It was found that by making use of two capillaries of different diameters an apparatus could be constructed which would give very good results and which would require not more than about 2 cc. of organic liquid. A detailed description of this apparatus will be given in another paper.

The thermostat employed was an ordinary rectangular brass box of about 15 liters capacity, fitted with plane glass sides. It was equipped with the usual heating and cooling coils. The mercury regulator held the temperature constant to within $\pm 0.03^{\circ}\text{C}$. The stirrer was equipped with a convenient switch so that it could be turned off at the moment of making a reading through the telescope of the cathetometer. The vibration of the stirring apparatus often causes such agitation of the liquid surfaces that the position of the wide meniscus cannot be accurately determined.

A 75-watt lamp mounted behind a ground glass screen was used as the source of illumination. A mirror, 2 x 18 inches, was set at an angle of 45° to a line passing through the center of the telescope. It also made an angle of 45° with the ground glass plate which was parallel to the line of sight through the telescope. This method of illuminating the wide menisci was compared with the methods used by Richards and Coombs² and by Harkins and Humphrey.³ The results obtained were found to agree closely with those obtained by the other methods.

Purification of Liquids. One of the principal sources of error in the determination of surface tension and of interfacial tension values is the presence of capillary-active impurities in the liquids. Thus, strongly adsorbed substances need be present only in infinitesimal amounts in order to produce a marked change in interfacial tension. The capillary height method of measurement used in this work is particularly susceptible to this effect. This method

¹ J. Am. Chem. Soc., 50, 1961 (1928).

² J. Am. Chem. Soc., 37, 1643 (1915).

³ J. Am. Chem. Soc., 38, 242 (1916).

is much more static than other methods, such as the drop volume or maximum bubble pressure methods, and impurities have more time to diffuse through the liquid and collect at the interface. For this reason, special care was taken in the purification of all liquids used. With one exception, namely toluene, the standards of purity attained are as high as any previously reported in the literature. The physical constants of all the liquids used had been precisely defined by one or more investigators. The following constants were selected for comparison with the best values in the literature as an indication of the purity of the compound. These values were checked within the limit of the experimental error in every case except the one previously noted.

Density. Specific gravity bottles of 25 and 50 cc. capacity with very finely ground glass joints were used in these determinations. The temperature of the measurement was determined with a thermometer certified by the U. S. Bureau of Standards and the variation was not more than $\pm 0.02^{\circ}\text{C}$. The volume of the specific gravity bottles at each temperature was determined by finding the weight of water contained and dividing by the known density of pure water at that temperature. This gives the absolute volumes reduced to 3.98°C .

The density was found in general to be the most sensitive criterion of purity. Other properties in special cases are better indicators of the presence of small amounts of impurities, but the density is the best single standard. In this connection it should be emphasized that no single physical property is a reliable measure of the chemical homogeneity of a given liquid. Unless the nature and specific effect of the impurities are known, several different and characteristic properties must be determined.

Boiling Point. The boiling points were determined with precision thermometers having a total range of fifty degrees, graduated in tenths of a degree, and calibrated by the Bureau of Standards except where otherwise noted. Every precaution necessary for obtaining the true ebullition temperature was observed and all corrections applied. All values have been reduced to a barometric pressure of 760 mm. of mercury. Where no values of dp/dt in the neighborhood of the boiling point were available, the integrated form of the Clausius-Clapeyron equation was used for the calculation. It was realized that a correct and constant boiling point is one of the least reliable tests of purity. Therefore, the closely checking results obtained for every liquid investigated were not necessarily considered to be proofs of a high degree of purity.

Freezing Points. Because many of the liquids employed in this research have very low freezing points, it was not practical to make these determinations in all cases. The values recorded agree very closely with the best of those reported by other workers, and this may be taken as a quite reliable indication of the absence of impurities.

The liquids used in addition to water were benzene, toluene, ethylbenzene, *n*-butylbenzene, nitrobenzene, chlorobenzene and dimethyl aniline.

The methods of purification are indicated in Tables I and II. The boiling points, freezing points and density values are given and are compared with a few of the best values from the literature.

The water used throughout these experiments was of conductivity grade. Care was taken to remove all traces of dissolved air and carbon dioxide in order to avoid any changes in density due to the presence of dissolved gases.

TABLE I

Substance	Method of Purification	Boiling Points °C		Authors
		Found	Other Values	
Benzene	Method of Richards and Shipley ¹	80.20	80.20	Young
		80.21	80.20	T. & M. (1926)
Toluene	Same as above without recrystallization	110.72 ± .01	110.7	Young
			110.6	Perkin
			110.46	R. & S. (1916)
			110.80	T. & M. (1926)
Ethylbenzene	Hg, CaCl ₂ , P ₂ O ₅ fr. two times	136.11 ± .01	136.18	Young & For- tey
			136.1	Mathews
			135.98	Richards & Barry
			136.15	T. & M. (1926)
Butylbenzene	Same as toluene	183.46 ± .10	183.10	T. & M. (1928)
			180.	Reed & Foster
Nitrobenzene	HCl, K ₂ CO ₃ , steam dist., fr., vac. fr. twice, cryst. twice	210.9 ± .1	210.8	Perkin
			209.2	Friswell
			210.60	Louguinine
Chlorobenzene	NaOH, steam dist. twice, CaCl ₂ , P ₂ O ₅ , fr.	132.01 ± .01	132.00	Young
			131.7	Mathews
			132.00	T. & M. (1926)
Dimethyl-aniline	Method of Nelson and Wales ²	194.	193	Perkin
			193.1	Kahlbaum
			192.68	Louguinine

¹ Richards and Shipley: J. Am. Chem. Soc., **38**, 989 (1916).

² Nelson and Wales: J. Am. Chem. Soc., **47**, 867 (1925).

TABLE II

Substance	Freezing Points °C		Temp.	Densities (Ref. to 3.98°C)		Author
	Found	Other Values		Found	Other Values	
Benzene	5.49 ± .01	5.58	25.13°	0.8732	0.8736	Young
	5.50 ± .01	5.50			0.87351	T. & M. (1926)
		5.493				R. C. & S.
					0.87344	Patterson
					0.87329	Int. Crit. Tab.
			20.15°	0.8783	0.8788	R. & S. (1924)
					0.8790	R. & S. (1916)
					0.8788	Young
					0.87862	Int. Crit. Tab.
					0.87879	T. & M. (1926)
Toluene			25.13°	0.8610	0.86225	T. & M. (1926)
					0.8624	Perkin
					0.8610	Int. Crit. Tab.
Ethyl- benzene			25.13°	0.8625	0.8624	Int. Crit. Tab.
					0.86239	T. & M. (1926)
Butyl- benzene			25.13°	0.8561	0.8563	T. & M. (1928)
Nitro- benzene	5.60 ± .02	5.67				Tamman
		5.7				Hansen
		5.82				Louguinine
			25.13°	1.1980	1.1972	Walden
					1.1983	Perkin
					1.1981	Int. Crit. Tab.
			15.13°	1.2078	1.2080	Int. Crit. Tab.
Chloro- benzene			25.13°	1.1008	1.1008	T. & M. (1926)
					1.1010	Perkin
Dimethyl- aniline	2.25 ± .02	2.5				Menschutkin
		1.96				Ampoula & Rimatoni
			25.13°	0.9518	0.9518	Int. Crit. Tab.

References to the Literature giving the preceding physical constants are listed below:

- | | |
|--|--|
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| Bramley | J. Chem. Soc., 109, 454 (1916). |
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| Kahlbaum | Z. physik. Chem., 26, 606 (1898). |
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| Perkin (1896) | J. Chem. Soc., 69, 1191 (1896). |
| Reed and Foster | J. Am. Chem. Soc., 48, 1606 (1926). |
| Richards and Barry | J. Am. Chem. Soc., 37, 998 (1915). |
| Richards, Carver and Schrumb | J. Am. Chem. Soc., 41, 2019 (1919). |
| Richards and Shipley (1914) | J. Am. Chem. Soc., 36, 1825 (1914). |
| Richards and Shipley (1916) | J. Am. Chem. Soc., 38, 989 (1916). |
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| Timmermans and Martin | J. Chim. phys., 23, 747 (1926). |
| | J. Chim. phys., 25, 411 (1928). |
| Walden | Z. physik. Chem., 65, 141 (1908). |
| Young | Sci. Proc. Roy. Dublin Soc., N. S. 12, 374 (1910). |
| Young and Fortey | J. Chem. Soc., 83, 45 (1903). |
| International Critical Tables, III, p. 27. | |

General Procedure

The solutions of the organic liquids were made up in all cases by weight. A few of the more dilute solutions were prepared by the method of successive dilutions, but, in general all the mixtures were made up directly from the pure liquids. They were preserved in Pyrex flasks having tightly-fitting ground glass stoppers. In most cases the interfacial tensions were determined within a few days after the solutions were made up. Errors due to changes in concentration by preferential evaporation of the more volatile constituent of the mixture were thus kept at a minimum.

With the Bartell-Miller method the densities need not be determined with extreme accuracy. A reasonable amount of care is necessary, however, in order to cut down the probable error from this source to a negligible quantity. Accordingly the densities of each pure liquid and every mixture were determined in 25 cc. or 50 cc. specific gravity bottles. It is believed that these determinations are not in error by more than ± 0.0002 units.

With the double capillary method used by us the density measurements are of decidedly secondary importance. Accordingly the densities of the mixtures were calculated from the volume percent of each constituent present. The density of a mixture of approximately 50 percent composition was determined. This has been shown to represent the maximum deviation from the calculated density.¹ By interpolation the increase in density on mixing could then be determined for each mixture. These corrections very seldom caused any change in the calculated value of the interfacial tension.

Mixtures of nitrobenzene were made up with each of the four hydrocarbons, benzene, toluene, ethylbenzene and butylbenzene. The interfacial tensions of these solutions against water at 25° C were determined throughout

¹ Brown: J. Chem. Soc., 39 (1881); Linebarger: Am. Chem. J., 18, 429 (1896).

the concentration range. The interfacial tension values of the mixtures with benzene were also determined at 15° and 35° C; those with toluene at the additional temperatures of 15°, 30° and 35° C. Mixtures of dimethyl aniline were made up with benzene, and the interfacial tension values throughout the concentration range were determined at 35° C. The values obtained at 25° are given in Tables III and IV. The final results calculated from data obtained at the other temperatures are given in Table IX.

The amount of nitrobenzene adsorbed at the interface has been calculated by means of the modified Gibbs equation, $q = -\frac{1}{RT} \frac{d S_{23}}{d \ln c}$. The adsorption from the different solvents is found to increase in the order benzene < toluene < ethylbenzene < butylbenzene; this would be expected from the relative differences between the interfacial tensions of the pure liquids. Because the solutions are so nearly "ideal" it is felt that the adsorption equation should hold reasonably well up to concentrations of fifty percent, at which concentration the maximum value of q is very nearly reached. At higher concentrations the equation obviously fails to express the true values. This will be discussed more fully in a later section.

TABLE III
Adsorption of Nitrobenzene
From Benzene Solution at 25.13°C

Concentration of Solution mols/cc $\times 10^{-3}$	Nat. Log. Concentration $\ln C-10$	Interfacial Tension dynes/cm.	Slope $\frac{d S_{23}}{d \ln c}$	Amt. Adsorbed q mols/cm ²
0.0000	—	34.70	0.000	0.0000
0.3355	2.0001	33.80	0.834	0.3364
1.0020	3.0942	32.60	1.360	0.5484
2.485	4.0025	30.67	2.80	1.129
3.877	4.4473	29.32	3.46	1.395
5.742	4.8400	27.79	4.13	1.666
7.480	5.1044	26.70	4.20	1.694
9.736	5.3680	25.73	4.20	1.694

From Toluene Solution at 25.13° C

0.0000	—	36.30	0.000	0.0000
0.2899	1.8506	35.50	0.822	0.3315
0.4718	2.3411	35.06	1.170	0.4719
0.5678	2.5263	34.06	1.410	0.5685
1.440	3.4569	33.38	2.133	0.8602
2.440	3.9686	31.86	3.668	1.479
6.120	4.9039	28.00	4.605	1.858
9.736	5.3680	25.73	4.780	1.928

TABLE IV
Adsorption of Nitrobenzene
from an Homologous Series of Hydrocarbons at
25.13° C Ethylbenzene Solution

Concentration of solution mols/cc $\times 10^{-3}$	Nat. Log. Concentration lnC-10	Interfacial Tension dynes/cm.	Slope $\frac{d S_{21}}{d \ln c}$	Amt. Adsorbed q mols/cm ²
0.0000	—	38.39	0.000	0.0000
0.1974	1.4698	37.77	0.464	0.1871
0.3652	2.0850	37.18	1.440	0.5808
0.7264	2.7726	36.04	1.834	0.7396
1.807	3.6839	33.73	3.446	1.390
3.515	4.3492	31.15	4.214	1.699
5.916	4.8698	28.94	4.570	2.206
9.736	5.3680	25.73	5.918	2.387

Butyl Benzene Solution

0.0000	—	41.58	0.000	0.0000
0.1968	1.4464	40.23	0.671	0.2706
0.5252	2.4517	38.51	2.553	1.030
1.604	3.5647	35.00	3.916	1.579
3.107	4.2259	32.03	4.770	1.924
6.246	4.9241	28.48	6.135	2.474
9.736	5.3680	25.73	6.355	2.563

Tests of the Validity of the Mixture Law. For this purpose the chlorobenzene-benzene system was selected. These liquids form practically an "ideal solution"; there is no appreciable volume change or heat effect on mixing. One would expect benzene with its lower interfacial tension to be positively adsorbed. Owing to its non-polar nature, however, its preferential adsorption is small. Unfortunately for our purpose the density and molecular weight ratios are not greatly different, so that the calculated difference between the volume fraction and mol fraction of the mixture is small.

Using the mol fraction units several experimental points on the interfacial tension-concentration curve were found to fall slightly above the straight line curve. The differences are so small, however, that they could be attributed to experimental errors. When weight fraction units are used the resulting interfacial tension-concentration curve falls far below the calculated values. When volume fraction units are used a curve is obtained which is slightly below the straight line curve indicating a slight preferential adsorption of benzene as would be expected. The results of these tests are then most favorable to the use of the volume fraction units; they are not, however, to be regarded as being conclusive. Further confirmation of the correctness of these units was obtained in data presented in Table VI, the significance of which will be discussed later. It is evident, however, that the use of volume fraction units yields results which agree among themselves much better than those obtained by the use of mol fraction units.

TABLE V

Test of Validity of Mixture Law
Method of Maximum Limiting Concentration
Chlorobenzene-Benzene System at 25°C

Interfacial Tension dynes/cm.	Weight Fraction Benzene	Mol Fraction Benzene	Volume Fraction Benzene	Limit Fraction Calc. from mixture law
36.72	0.2628	0.3392	0.3099	0.378
35.98	0.5480	0.6359	0.6045	0.607
35.33	0.7577	0.8183	0.7975	0.810
34.96	0.9016	0.9296	0.9204	0.926
34.74	0.9636	0.9745	0.9709	0.992

Correction for Molecules normally present in the Surface.—Langmuir¹ and others have made calculations of molecular area by employing maximum adsorption values of q (i.e. q_L values) and substituting in the equation $a = 1/Nq_L$. These calculations have been based upon data obtained from comparatively dilute solutions.

It appears that this method should not be extended to the more concentrated solutions without taking into account the molecules of adsorbate normally present in the interface before adsorption occurs. The number of molecules thus present in one square centimeter of surface has been assumed to be equal to the two-thirds power of the number of molecules contained in one cubic centimeter of the solution. If c is expressed in mols per cm^3 then $(cN)^{2/3}$ gives the number of molecules lying in a plane of 1 cm^2 area within the solution. This term divided by N (or $c^{2/3}/N^{1/3}$) will give the number of moles per cm^2 initially present in the surface layer.

The total concentration of the interfacial layer should thus be equal to the sum $(q + c^{2/3}/N^{1/3})$.

In the dilute concentration range considered by Langmuir the factor $c^{2/3}/N^{1/3}$ is very small in comparison with q , and lies within limits of the experimental error in determining the latter value. Consequently, it need not be considered in these cases. Harkins and Wampler² have recently shown that in several cases the correction becomes appreciable at concentrations of approximately 1 mol per liter. In our work the value of $c^{2/3}/N^{1/3}$ was usually found to be one to two times greater than that of q . Mathews and Stamm made use of the limiting slope method for calculating the area of molecules adsorbed from concentrated solutions but they did not take into account the molecules initially present in the interface. It would seem that this method of calculation should give results several times too large.

An attempt was made in our work to obtain evidence bearing upon this point.

¹ J. Am. Chem. Soc., 39, 1848 (1917).

² J. Am. Chem. Soc., 53, 850 (1931).

TABLE VI
Molecular Area of Nitrobenzene
Adsorption from Butylbenzene Solution at 25° C.

Fraction Nitrobenzene	Slope $\frac{d S_{N_2}}{d \ln c}$	Mol Fraction Concentration Units					Molecular Area $\text{cm}^2 \times 10^{-16}$		
		Interface Concentration		Thickness of Film $\text{cm.} \times 10^{-7}$		Max. Ads. (c)	from Thickness		
		Total mols/cm ² $\times 10^{-3}$	Excess mols/cm ² $\times 10^{-3}$	Adsorbed mols/cm ² $\times 10^{-10}$ (a)	Total mols/cm ² $\times 10^{-16}$		(a)	(b)	
0.0305	0.671	0.503	0.306	0.2706	0.6704		0.885	1.331	246.
0.0800	2.553	1.412	0.887	1.030	1.799		1.161	1.273	91.6
0.2313	3.916	2.878	1.274	1.579	3.198		1.339	1.111	51.6
0.4167	4.770	4.485	1.378	1.924	4.439		1.393	0.990	37.2
0.7318	6.135	7.04	0.79	2.474	6.481		3.14	0.992	30.6
1.000	6.355	9.736	—	2.563	7.949		—	0.817	30.6
							Average 14.9	18.4	Limit 30.6
0.0202	0.671	0.808	0.611	0.2706	0.6704		0.443	0.830	246.
0.0540	2.553	1.889	1.364	1.030	1.799		0.755	0.952	91.6
0.0647	3.916	4.055	2.451	1.579	3.198		0.645	0.710	51.6
0.3190	4.770	5.865	2.758	1.924	4.439		0.698	0.758	37.2
0.6415	6.135	8.04	1.79	2.474	6.481		1.382	0.807	30.6
1.00	6.355	9.736	0.00	2.563	7.949		—	0.817	30.6
							Average 24.8	22.0	Limit 30.6

The amount of adsorption at the interface was determined by measurement of interfacial tension and application of the Gibbs formulation. The amount of solute present at the interface at each concentration investigated was also calculated. The sum of these theoretically give the total amount of solute at the interface.

Data obtained for a given system are given in the form of a graph in Fig. 1. While the curves shown on this graph have been plotted from actual data, no units have been given in order that the figure may be considered as being applicable to a perfectly general case.

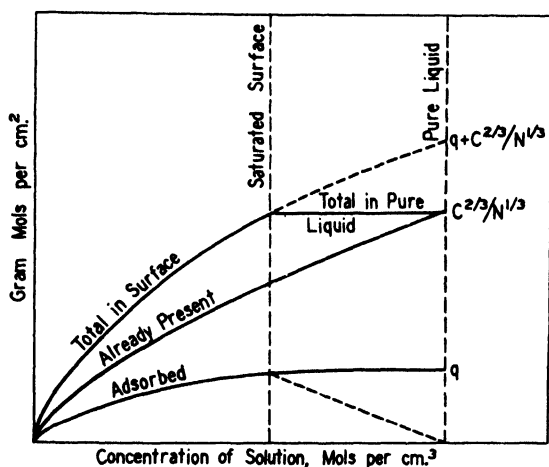


FIG. 1

Molecules of Adsorbate present at an Interface

The lower curve of Fig. 1 represents the number of mols adsorbed at the liquid-liquid interface, as calculated by means of the Gibbs equation. This equation is derived upon the necessary condition that the osmotic pressure within the solution be proportional to the mol fraction of the constituents present¹. For the nearly ideal solutions used in this work it has been assumed that this condition does hold up to a concentration of one mol per liter, and a close approximation of it may be expected to hold up to four or five times this concentration. This is about the concentration necessary for the production of a saturated surface layer of the solute molecules. In substantiation of the statement that the calculated values of q are approximately correct, it may be pointed out that q has very nearly attained its maximum value at that concentration which is in equilibrium with a saturated interface. Above this concentration the equation fails to express the true values of the adsorption. These deviations will be discussed later.

The middle curve of Fig. 1 represents the number of the solute molecules present in an unit surface assuming they are here randomly distributed as they are throughout a unit volume.

¹ Milner: Phil. Mag., (6) 13, 96 (1907).

Considering a plane in the interior of the solution it seems reasonable to expect that the number of molecules per square centimeter should be equal to the two thirds power of the molecules per cubic centimeter. If this plane be moved into the interface, the perfectly random distribution normally occurring in the bulk of the solution may be affected by the unbalanced surface forces. The solute molecules being, in general, quite polar in nature will be oriented in the interface even when no adsorption occurs. Then, unless the molecules are symmetrical in shape and uniform in all dimensions, the curve will not represent the true number of mols present in the interface.

The upper curve is given by the sum of the values of the two lower curves and represents the total number of molecules at the interface. The upper dotted portion of this curve represents imaginary values, since the surface concentration cannot exceed that found for the pure solute liquid. The calculated total number of mols in the surface is thus in error by at least the amount which is represented by the vertical distance between the dotted line and solid horizontal line representing the number of mols in a saturated surface. This error may be due to errors introduced in either or both of the component parts of the sum. By assuming the $c^{3/4}/N^{1/4}$ factor to be correct, a more probable value of q is obtained which is represented by the lower dotted line shown in the figure. This dotted line together with the first portion of the solid adsorption curve represent the maximum values of q . Ostwald and Izzaguirre¹ as well as several workers in this laboratory² have obtained results which indicate that the actual relative adsorption is practically always less than the theoretical maximum values except in the dilute solutions. They obtained an equation which appears to give the correct relative values throughout the concentration range. These corrections could not be attempted in this paper, since no method is available for the conversion of relative adsorption values to absolute values.

Summarization of Methods

In recapitulation the methods available for the determination of area of adsorbed molecules in interfacial films are as follows:

(1) Maximum adsorption method.

The maximum adsorption method as referred to in this paper is based upon the limiting slope method used by Langmuir. In case c (the concentration of solute in bulk) is not negligible in comparison to c' (the concentration of solute in the interface) the number of molecules of solute normally present in the interface before adsorption must be taken into account, the total number of molecules at the interface being represented by the formulation $q_L + c^{3/4}/N^{1/4}$. In the maximum adsorption method the total number of molecules were considered. It follows then that the limiting slope method used by Langmuir and the "maximum adsorption" method used by us differ only by the factor $c^{3/4}/N^{1/4}$ which was used in the latter method.

¹ Kolloid-Z., 30, 279 (1922); 32, 57 (1923).

² Bartell and Sloan: J. Am. Chem. Soc., 51, 1643 (1929); Bartell and Miller: Thesis (unpublished), University of Michigan (1929); Bartell and Scheffler: J. Am. Chem. Soc., 53, 2507 (1931); Bartell, Scheffler and Sloan: 53, 2501 (1931).

The values of the molecular areas as calculated by this method are given by the reciprocal of the corrected total number of molecules per cm^2 in the interface. This treatment has been previously discussed in connection with Fig. 1.

(2) The mixture law method as used by Mathews and Stamm has been fully discussed and needs no further amplification at this point.

(3) The modified mixture law method has also been discussed. The difference between this method and the mixture law method is brought out by the emphasis given below.

$$\begin{array}{ccc}
 \text{Mixture Law Method} & & \text{Modified Mixture Law Method} \\
 \text{(a)} & & \text{(b)} \\
 t = \frac{\text{mols adsorbed}/\text{cm}^2}{\text{mols adsorbed}/\text{cm}^3} & = & \frac{\text{total mols in surface}/\text{cm}^2}{\text{total mols in surface}/\text{cm}^3} \\
 = \frac{q}{c' - c} & = & \frac{q + c^{3/2}/N^{1/2}}{c'}
 \end{array}$$

In Tables VII and VIII both of these methods have been used in calculating the molecular area of nitrobenzene, the different columns being labeled as above, (a) and (b) respectively. The third column (c) contains data obtained by the maximum adsorption method. In the same way, a number of other values of the molecular area of nitrobenzene and dimethyl aniline have been calculated. These results are summarized in Table IX.

For the purpose of comparison with the data of Mathews and Stamm a fourth column (d) is included in the table, the values of which have been calculated according to the original limiting slope method when applied to concentrated solutions. It is seen that these values are much too high.

TABLE IX
Molecular Area of Nitrobenzene and of Dimethyl Aniline
A Summary of Values

Nitrobenzene Solvent	Temperature	Area per Molecule $\text{cm}^2 \times 10^{-16}$			
		(a)	(b)	(c)	(d)
Benzene	15°	30.2	23.0	30.4	94.8
"	25°	34.6	23.2	30.6	97.4
"	35°	33.3	22.5	30.8	100.5
Toluene	15°	34.5	21.7	30.4	88.5
"	25°	29.7	21.5	30.6	85.7
"	30°	32.4	21.5	30.7	87.1
"	35°	33.4	20.9	30.8	87.8
Ethylbenzene	25°	27.2	20.6	30.6	69.1
Butylbenzene	25°	24.8	21.0	30.6	64.5
Average		32.1	21.8	30.6	86.2
<i>Dimethyl Aniline</i>					
Benzene	35°	41.5	26.6	35.5	111.3
Benzene (Mathews & Stamm)	25°	38.4	—	—	75.8
Heptane (" " ")	25°	34.8	—	—	57.2

a = area calculated from mixture law method.

b = " " " modified mixture law method.

c = " " " maximum adsorption method.

d = " " " limiting slope method.

Discussion of Results

The results presented in column (a) calculated by the mixture law method are subject to the errors inherent in the modified Gibbs adsorption equation and to the errors inherent in the mixture law method as used.

The results presented in column (b) calculated by the modified mixture law method are subject to all the errors of the previous method and in addition to the error involved in the assumption that the number of molecules originally present in unit area within the bulk of the solution is the same as the number present in unit area of surface.

This method has the advantage over the original mixture law method in that the data obtained throughout the entire concentration range may be used, while the original mixture law method is limited in application to points on the first portion of the interfacial tension-concentration curve in which the maximum adsorption values have not been reached. It is in this range that the absolute values of the quantities from which the molecular areas are calculated are relatively small. Hence, the same experimental error produces a much larger relative error in the calculated value of area than would be obtained by use of the other method. At very low concentrations, low values are obtained by the second method. This may be the result of the relatively stronger tendency for the molecules near the surface to become oriented. The spatial distribution of the molecules will then be furthest from a random arrangement and the calculated value of the factor $c^{2/3}/N^{1/3}$ will be in error by a greater amount with these dilute solutions.

The results obtained in column (c) calculated by the maximum adsorption method show good agreement with the values in column (a). The reliability of the results calculated by the maximum adsorption method is subject to question. This method cannot be considered to compare in accuracy with the similar limiting slope method when this latter method is applied to dilute solutions of highly adsorbable substances. It is evident that the *final* limit value obtained by the maximum adsorption method is independent of the amount of adsorption, and depends only upon the molecular volume of the adsorbed molecules. The arbitrary manner of this calculation throws considerable doubt upon the validity of the results.

None of the methods above described for determining the area of adsorbed molecules are entirely free from theoretical objections. The values obtained and presented in this paper agree among themselves more closely than have values previously obtained by similar methods.

The methods which have so far been used in this paper for the calculation of molecular area, while widely different in many respects, are alike in that they all depend upon surface film measurements. Comparison with the values obtained by Stewart from the totally different method based upon the measurement of the diffraction of x-rays by liquids should provide a good test of the absolute accuracy of the surface film methods. At the same time it furnishes an independent check of the theory of cybotaxis upon which Stewart's calculations of molecular diameter are based. Investigations by the

x-ray diffraction method upon the particular liquids used by us are not available, but values for a sufficient number of substitution products of benzene have been worked out by Stewart to enable one to draw quite definite conclusions as to the most probable dimensions of nitrobenzene and dimethyl aniline. The values given in Table X are compatible in every case with the results presented in this paper.

TABLE X
The Area of Molecules calculated from Stewart's Values
for the Molecular Diameters

Substance	Molecular Diameter cm. $\times 10^{-8}$	Molecular Area sq. cm. $\times 10^{-16}$
Benzene	4.70	31.4
Toluene	5.06	34.8
Ethylbenzene	4.99	40.6
Isopropylbenzene	5.36	43.0
Phenol	4.77	30.8
Aniline	4.75	31.8

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MONOMOLECULAR FILMS.* THE SOLID-LIQUID INTERFACE AND THE SEDIMENTATION AND FLOCCULATION OF POWDERS IN LIQUIDS¹

BY WILLIAM D. HARKINS AND DAVID M. GANS

1. Introduction

While it is known that the volume occupied by a powder after long settling in a liquid is dependent upon the composition of the liquid, the fact that monomolecular films at the solid-liquid interface have an extremely great effect upon the settling, has been unknown. The phenomena of settling in both aqueous and organic liquids present many remarkable characteristics. While experiments in both classes of liquids have been carried out, the present paper will be restricted almost entirely to those of the organic type, since they have revealed more new unknown and striking relations.



The energy of immersion of a solid oxide in the form of a powder, is very low in a non-polar liquid such as hexane, benzene, or carbon tetrachloride. However, if a small amount of an organic acid, alcohol, amine, or any other similar liquid is added to the non-polar liquid, the heat of immersion is greatly increased. Molecules of this type may be designated as hetero-homopolar, and are commonly represented by the symbol, in which the circle represents a polar group.

The data² on the heat of immersion indicate that the polar groups in such molecules are oriented toward the oxide at the solid-liquid interface. Obviously the orientation of the molecules in this interface is the same with a pure liquid if its molecules are of the hetero-homopolar type.

The purpose of the work reported in this paper was to determine the effect of such an oriented interfacial film upon the final sedimentation, and upon the rate of sedimentation of a powder in the liquid, since these indicate the extent of the flocculation of the powder.

The presence of a monomolecular oriented film of molecules of the hetero-homopolar type is found to prevent flocculation. If, however, molecules of water are also present, the mixed film does not prevent flocculation to the same extent, and if a considerable amount of water is present in the mixed film, the flocculation may be as great as in a pure non-polar liquid.

* Contribution from the George Herbert Jones Laboratory of the University of Chicago.

¹ This research was made possible by the support of the Titanium Pigment Co., Inc.

² Harkins and Dahlstrom: Ind. Eng. Chem., **22**, 897 (1930).

2. Experimental Procedure

In a part of the experiments on settling, about 48 cu. cm. of the liquid was drawn by a syphon into a graduated glass-stoppered cylinder and the stopper immediately inserted. The powder, dried at a high temperature in a high vacuum (8.0 grams), was then weighed quickly and poured into the cylinder, exposing the powder to the atmosphere as little as possible. The tightly stoppered cylinders were then vigorously shaken and allowed to stand, and the volume of the suspension read off at intervals. Other more carefully conducted experiments were carried out in sealed pyrex tubes, with special care to exclude moisture, and were used to check the values obtained in the stoppered cylinders.

3. Preparation of Liquids and Drying of Powder

The titanic oxide (TiO_2) used in this work was dried in a high vacuum at 450° to 550°C . for 24 hours.

Thiophene-free benzene was dried over oil-free sodium wire for several weeks. Fresh sodium wire was added several times during this period. The benzene was then distilled, and the distillate stored with a large quantity of sodium wire distributed through its volume.

The oleic acid was purified by the method used by Harkins and Beeman, who applied the well known procedure in which the lead and barium salts are used.

4. An Adsorption Method for the Determination of the Area of the Surface of a Powder

In problems which concern settling or those which involve surface energy it is of fundamental importance to have a method for the accurate determination of the area of the surface of a powder. The method described below is believed to be the most accurate of all known methods.

The powder, titanic oxide or silicon dioxide for example, is dried in a high vacuum at as high a temperature as can be used without affecting the area of the crystals. The cool, dry powder is immersed in a solution of oleic acid, butyric acid, or some other suitable acid, in *very dry* benzene, and the suspension is shaken until equilibrium is attained. After the powder has settled, a sample of the supernatant liquid is drawn off. The benzene of this sample and of a sample of the initial solution is evaporated off, if oleic acid is the solute chosen, and the oleic acid left from each solution is dissolved in 95% ethyl alcohol. The alcohol solutions are then titrated with carbonate free sodium hydroxide dissolved in water. The difference in concentration is considered to give the amount of acid adsorbed by the surface of the powder. Only air that is thoroughly dried by phosphorus pentoxide is allowed to enter the vessel in which the solution is prepared and the flasks in which the adsorption experiments are carried out.

Fig. 1 shows how the amount of oleic acid adsorbed by the surface of one gram of powder varies with the concentration of the final or equilibrium solution of oleic acid in benzene. At concentrations above 0.01 to 0.02 moles per

kilogram of benzene the adsorption becomes practically constant, and the oleic acid on the surface of the grains of powder may be said to form a condensed film. There is some evidence which seems to indicate that this film is monomolecular.

It may be assumed that the area occupied per molecule of oleic acid at the interface is 20.0 sq. Å., which is about the mean value for condensed films of the acid on water at a zero film pressure. On this basis, the area for $\text{TiO}_2\text{-I}$ was calculated as 22.9 sq. m. per cu. cm. of titanic oxide (3.89 grams), and for $\text{TiO}_2\text{-II}$ as 14.4 sq. m. per cu. cm. The ratio of these values is 1.59, while

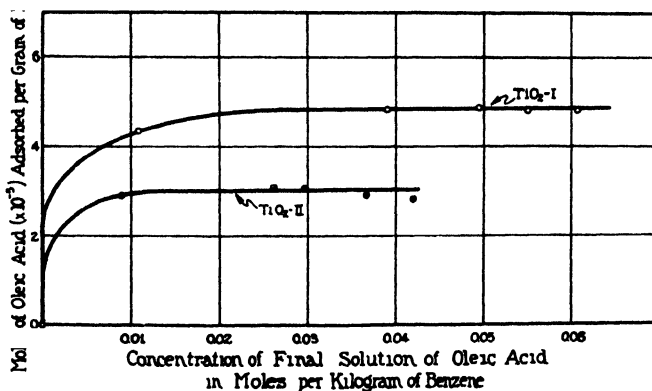


FIG. 1
Adsorption Curves for Dried Titanic Oxides

the ratio for the energy of immersion¹ in ethyl acetate for the same two powders is 1.53, and the ratio of the weights of propyl alcohol adsorbed² from the vapor per unit weight of these powders at a constant vapor pressure equal to one-half the saturation pressure at room temperature is 1.53.

The area of the powder $\text{TiO}_2\text{-II}$ as determined for us by a microscopic method,³ applicable to crystalline powders, is 13.8 sq. m. per cu. cm. of powder, on the assumption of a spherical shape for each particle. This is in good agreement with the value 14.4 obtained by the adsorption method. However, the closeness of the agreement is to some extent accidental, since the adsorption method includes the area of the colloidal part of the powder, which is not included by the microscopic method. However the agreement between the two methods indicates that the film is monomolecular and not polymolecular.

5. Effect of a Monomolecular Film of Oleic Acid on the Settling of Powders

The experiments described below show that if a powdered crystalline oxide is allowed to settle in benzene the most complete settling is obtained when the grains of powder are coated with a film of oleic acid one molecule thick.

¹ Harkins and Dahlstrom: to be published.

² Gans and Brooks: to be published.

³ Dunn: Ind. Eng. Chem., Anal. Ed., 2, 59 (1930).

A fine powder consisting of titanic oxide was dried in a high vacuum at 400°C . for 24 hours. It was then suspended in extremely dry benzene in tubes about 2 cm. in diameter. After several weeks the top of the powder attained a constant level. The density of the titanic oxide used is 3.89, and so 3.89 g. of the material may be considered to occupy 1 cu. cm. This amount of material was found to settle to a final volume of 20 cu. cm., that is, until 19 of the 20 cu. cm. was occupied by the benzene between the grains of powder.

It was found that if oleic acid was added to the benzene any *initial* concentration up to 0.005 moles per kg. of benzene produced no great effect

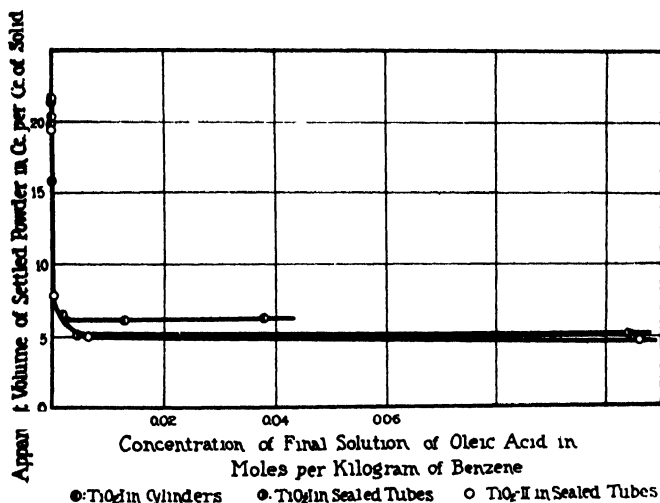


FIG. 2
Variation of Extent of Settling with Amount of Oleic Acid present

(Table I) but a concentration of 0.008 moles of oleic acid gave complete final settling to a volume of 6.5 cu. cm. per cu. cm. of oxide, while if the suspension were kept very dry in sealed tubes as little as 0.0016 moles per kg. of benzene produced settling to 16 cu. cm. per cu. cm. of material with sample I and to 8 cu. cm. with sample II.

It has been shown in Section 5 that oleic acid is highly adsorbed from its solutions in dry benzene by titanic oxide. Harkins and Dahlstrom¹ have shown that this is an effect which is obtained whenever an active or polar group is present in the organic compound used.

The values for the heat of immersion show that the active group is oriented toward the oxide and the non-polar group toward the benzene (Fig. 6). They show that powders which consist of the oxides of silicon, tin, zinc, or of barium sulfate, also exhibit a similar great adsorptive effect.

The effect of the adsorbed film of oleic acid in producing settling is shown by Fig. 2.

¹ Harkins and Dahlstrom: Ind. Eng. Chem., 22, 897 (1930).

Thus the presence of 0.005 moles of oleic acid per kg. of benzene in the *final* equilibrium solution is sufficient to cause complete settling, in which the initial volume of 21.5 cu. cm. per cu. cm. of solid in dry benzene alone is reduced by four times to 5 cu. cm. The addition of larger amounts of oleic acid is seen to produce no further effect.

TABLE I

Apparent Volume of Powder after Settling in Benzene
in Cc. per Cc. of Solid

C = Initial Concentration of Oleic Acid
Moles per Kg. Benzene

A. $\text{TiO}_2\text{-I}$ in Cylinders

% Water in the Powder	C = 0.0000	0.00145	0.00498	0.00827	0.0208	0.0490
0.00	20.3	21.5	19.8	6.5	6.1	6.3
0.05	20.6	21.9	21.1	20.8	20.1	20.0
0.25	20.7	22.0	22.1	21.6	21.7	21.5
0.95	22.2	22.1	22.1	21.6	21.8	22.0

B. $\text{TiO}_2\text{-I}$ in Sealed Tubes

% Water in the Powder	C = 0.0000	0.0016	0.0111	0.0354	0.1111
0.00	21.4	15.8	5.1	—	5.2
0.05	—	—	—	—	14.4
0.33	—	—	—	18.9	—

C. $\text{TiO}_2\text{-II}$ in Sealed Tubes

% Water in the Powder	C = 0.0000	0.0016	0.0111	0.0354	0.1111
0.00	19.4	7.9	5.0	—	4.7
0.05	—	—	—	—	9.4
0.33	—	—	—	14.1	—

The curves of Section 4 indicate that at 0.005 moles per kg. of benzene the adsorbed film of oleic acid is monomolecular, but is not quite tightly packed.

It may be noted that the above refers to titanic oxide I or II when extreme precautions were taken to keep both the benzene and the titanic oxide very dry, and when the suspension was kept in sealed pyrex tubes. When the suspension was kept in glass stoppered cylinders an even smaller *final* concentration of oleic acid (about 0.003 moles per kg. of benzene) gave complete settling, but to only 6 instead of 5 cu. cm. That is, the minute amount of water which evidently was able to get into the film, decreased the amount of oleic acid necessary in the film and decreased the settling only slightly. It will be shown in Section 7 that slightly larger amounts of water in the oleic acid film decrease the extent of settling much more.

6. Effect of Vibration on Settling

The minimum volume for one cu. cm. of the solid after settling is found to be about 5 cu. cm. The density of titanic oxide is 3.89 so that the mass of one cu. cm. of the solid oxide is 3.89 grams. The volume of this amount of powder in the absence of the dispersing liquid was found to be 5.5 cu. cm. for $\text{TiO}_2\text{-I}$, and 3.5 cu. cm. for $\text{TiO}_2\text{-II}$, as obtained by a gentle pressure which was used to remove air pockets. The former of these values is approximately that obtained for the final volume of the suspension, while the latter value is considerably less.

In order to determine the effects of a gentle mechanical agitation upon the suspensions, the sealed tubes were put vertically in a large test tube rack which was caused to vibrate horizontally 400 times per minute with an amplitude of 1 cm. In order to increase the effect the tubes were left loose in the racks. After 30 hours of vibration almost no further settling occurred, but the vibration was continued until the total period was 64 hours.

TABLE II

Effect of Tapping on Apparent Volume of Powder
after Settling in Benzene

C = Initial Concentration of Oleic Acid
Moles per Kg. Benzene

A. Apparent Volume in Cc. per Cc. of Solid

% Water in the Powder	C = 0.0000	0.0016	0.0111	0.0354	0.1111
	1. $\text{TiO}_2\text{-I}$ in Sealed Tubes				
0.00	8.9	6.5	3.9	—	3.8
0.05	—	—	—	—	5.4
0.33	—	—	—	6.5	—
	2. $\text{TiO}_2\text{-II}$ in Sealed Tubes				
0.00	8.0	3.5	3.1	—	2.8
0.05	—	—	—	—	3.1
0.33	—	—	—	5.1	—

B. Ratio of Apparent Volume with Tapping to Apparent Volume without Tapping

	1. $\text{TiO}_2\text{-I}$ in Sealed Tubes				
0.00	0.42	0.41	0.76	—	0.73
0.05	—	—	—	—	0.38
0.33	—	—	—	0.27	—
	2. $\text{TiO}_2\text{-II}$ in Sealed Tubes				
0.00	0.41	0.44	0.62	—	0.60
0.05	—	—	—	—	0.33
0.33	—	—	—	0.36	—

The final volume (Table II) of the suspension was thus reduced to 8.9 and 8.0 cc. for TiO_2 -I and TiO_2 -II respectively in the absence of oleic acid, and to 3.9 and 3.1 cc. for an initial oleic acid concentration of 0.0111 moles per kg. of benzene.

Thus, when the particles are covered with a monomolecular film of oleic acid, the final volume of the suspension becomes in this way as small as or smaller than the volume of the dry powder. After the suspensions which contained oleic acid had settled to such a compact mass, it was very difficult to cause a resuspension of the powder by shaking, and to this extent the behavior is analogous to that known as the hard-settling of paints. For example, 45 minutes of vigorous shaking by hand was necessary to resuspend the powder in one of these tubes.

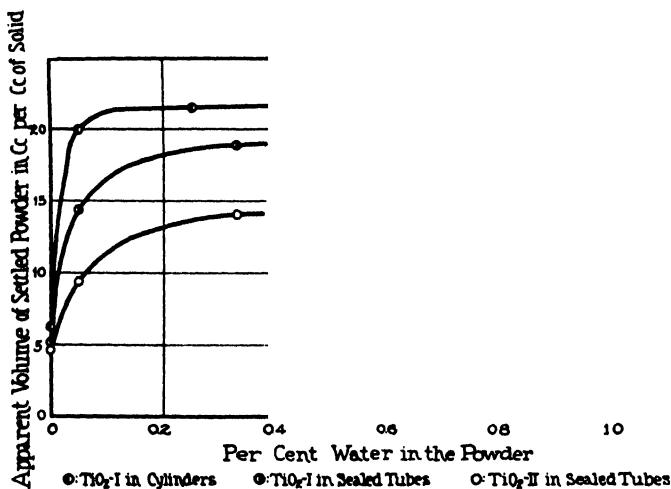


FIG. 3
Variation of Extent of Settling with Water Content of Powder

7. The Effect of Water

The results of certain experiments on the adsorption of oleic acid by titanic oxide have been given, but an important additional result obtained was that powder with a content of 0.05 per cent water, in the form of an adsorbed layer, adsorbs only about one-half as much oleic acid as the dry powder. Since the oleic acid film is very effective in producing settling of the suspension in benzene, it seemed important to determine the relation between the water content and the settling.

The results of the experiments are shown in Fig. 3. Powders which have settled completely to about 5 cu. cm. per cu. cm. of solid, are found to settle less and less as the water content is increased.

The data are summarized in Table I. In the first series (A) of experiments the presence of 0.33 per cent of water with the powder was sufficient to entirely

prevent the settling due to oleic acid, while in the second and third series the presence of this amount of water was found to overcome largely but not entirely the effect of the acid.

8. Texture of the Suspension

As has been shown, titanitic oxide in dry benzene gives a suspension with a large specific volume, about 21 cu. cm. per cu. cm. of solid. The suspension, after settling, is found to exhibit a coarse structure, sometimes with cavities one mm. or more in diameter. Such a suspension is often described as "floc-culated." If the suspension is shaken in the sealed tube, the walls of the glass become coated with a flocculated coating, which does not completely cover the glass, and which may be easily detached. Very little of the powder adheres to that part of the glass which is under the liquid.

An interesting phenomenon was observed with such tubes when the powder and dry benzene only were present. If such a sealed tube is shaken vigorously, it gives a spark over a gap of about 3 mm. if the glass is brought near the hand. Tubes in which oleic acid is also present were not found to exhibit this phenomenon, though it is possible that the glass becomes charged to a lesser extent.

When the suspension contains enough oleic acid to produce the maximum settling, the texture is very uniform and fine, and the suspension may be said to be deflocculated. With such suspensions the deposit on the glass is much more fine and more adherent.

If water is added, the suspension, after it is shaken, is found to be flocculated, and the structure of the suspension, with 0.33% of water to powder, is apparently the same as if neither oleic acid nor water were present.

9. Rate of Settling as influenced by the Composition of the Liquid

The rate of settling of the powder in benzene solutions which contain different amounts of oleic acid and of water is exhibited in Fig. 4. A striking feature of these curves is that in pure benzene the powder settles the most rapidly at first but settles least in 24 hours. If only oleic acid is added the rate of settling is often practically constant for an hour, and increases with increasing acid concentration up to a concentration of acid (about 0.01 m.) which gives a complete monomolecular film.

A peculiar relation is shown by curves b and c. The system is the same for both of these (0.0016 moles of oleic acid per kg. of benzene). Both settle at the same rate for three minutes and to the same extent in 24 hours. However, at the end of 3 minutes suspension c began to settle much more rapidly than b and departed very widely from the linear course of the latter.

Fig. 5 illustrates the behavior of a suspension in a tube which was called the "clock-tube," since it behaved with such regularity as to give a moderately accurate measure of an interval of time, four minutes in length, and also a shorter interval of about three minutes. The titanitic oxide contained 0.33 per cent of water, and was suspended in a solution which contained 0.035 moles of oleic acid per kg. of benzene. For four minutes the powder settled

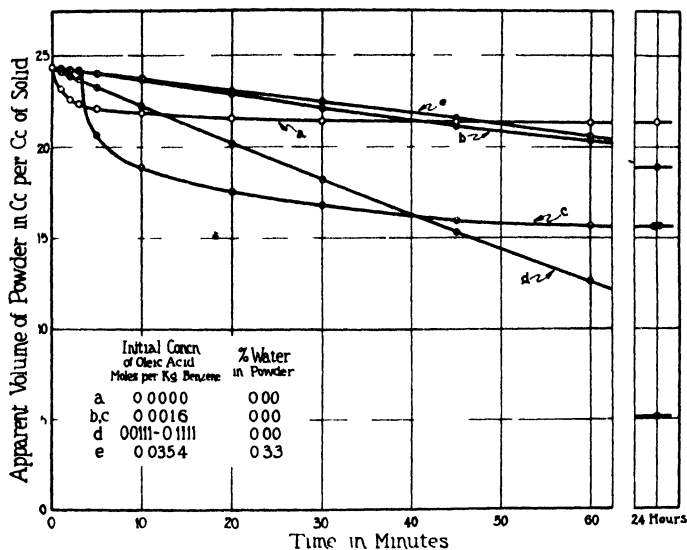


FIG. 4
Typical Settling Curves for Titanic Oxide

with extreme slowness (from 24.4 to 24.2 cu. cm.) at the top, and at the end of this period it fell with considerable rapidity from 24.2 cu. cm. at 4 minutes to 17 cu. cm. at 5 minutes and to 15 cu. cm. at 6 minutes, while in 60 minutes it fell only to 14 cu. cm. This procedure was repeated many times, after the tube was shaken.

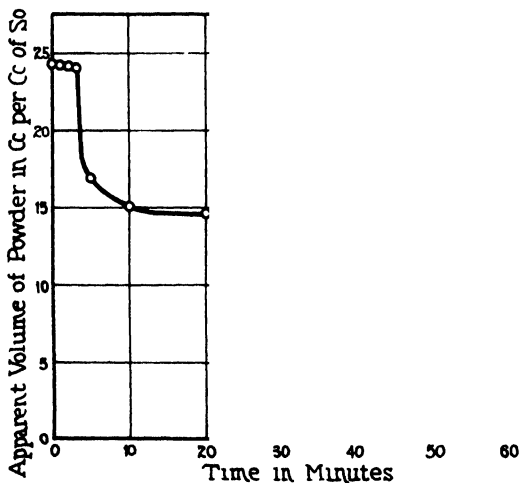


FIG. 5
Abnormal Settling Curve. Titanic Oxide plus 0.33% of Water,
Solution of 0.035 Moles Oleic Acid per Kg

At the beginning of the period the suspension appeared to be uniform (not flocculated), and this condition persisted for 4 minutes near the top of the cylinder, but only 3 minutes at the bottom. At the end of 3 minutes the suspension seemed to begin to settle suddenly and rapidly near the bottom of the tube. This settling drew the powder mostly away from a region about 2 cm. above the bottom of the tube. Then powder fell rapidly into this vacant space from just above, and by the continuation of this process the region almost free from powder moved rapidly up the tube.

The effect of *n*-butyric, *n*-heptylic, stearic and oleic acid on the rate of settling of graphite, barium sulphate, and silica gel in benzene, has been studied by Reh binder and his collaborators.¹ Their conclusions agree to a certain extent with those of the present paper, but a comparison is difficult since they studied the settling for about a one minute period only.

10. Orientation of the Molecules at the Interface Solid-Liquid

The values of the energy of immersion of oxides, such as titanite oxide, stannic oxide, silicon dioxide, and zinc oxide, indicate that in suspensions in benzene the carboxyl group of the acid is in all cases oriented toward the surface of the solid oxide (Fig. 6).

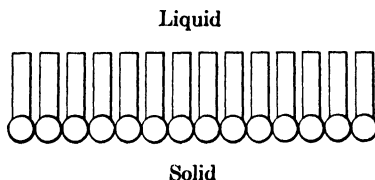


FIG. 6

Orientation of an Acid at the Surface of an Oxide

11. Discussion

It is often considered that the settling of powders in liquids and other related characteristics of the suspensions, are a simple function of the "wettability" of the powder by the liquid, but this is not true. Thus, it has been seen that dry titanite oxide in dry benzene settles only slightly. If oleic acid is present the heat of immersion is much higher, and the powder settles much more. However, the presence of small amounts of water in addition to the oleic acid may altogether prevent any additional settling due to the acid. The energy of immersion of the solid oxide in water, or in benzene which contains water, is even higher than the similar values with oleic acid. Thus the correlation of extent of settling with energy of wetting is not a simple one.

It has been seen, however, that the presence of a monomolecular film of oleic acid on the grains of powder greatly increases the amount of settling, and causes deflocculation. The effect might be ascribed to a lubricating action of

¹ Reh binder, Lagutkina, and Wenstrom: *Z. physik. Chem.*, **146 A**, 63 (1930).

the monomolecular film which does not allow one particle of powder to adhere to another. This behavior may be assumed to be due to the electrical relations at the interface solid-liquid. Thus the particles may be considered as more highly charged when oleic acid is present than when it is absent, but no evidence in favor of this conclusion has been obtained. The energy of adhesion and of cohesion may be involved as well. More work is being done on the various factors which affect the flocculation.

The writers wish to thank L. W. Ryan, Director of Research, Titanium Pigment Co., Inc., for many useful suggestions.

12. Summary

1. A monomolecular film of oleic acid on the surface of a fine powder suspended in a very dry non-polar liquid, is found to deflocculate the powder, and to cause it to settle to one-fourth the volume it would occupy if no oleic acid were present. This effect is produced by a monomolecular film of oleic acid even if it is not quite a condensed film. Thus the final volume occupied by 1 cu. cm. of titanic oxide in its flocculated suspension in pure and very dry benzene is 20 cu. cm., but this is reduced to 5 cu. cm. by a monomolecular film of oleic acid on each grain of powder.

2. Vibration of the tubes which contained the suspensions greatly reduced the above volumes to about 8 and 3 cu. cm. respectively. Thus the reduction in volume did not annul the great effect of the film of oleic acid.

3. The addition of very small amounts of water to the solution of oleic acid in benzene was found either to destroy entirely or else to remove partly the effect of the oleic acid in deflocculating the solution and causing settling to a small volume. Thus water caused the flocculation to reappear. The final volume of 1 cu. cm. of titanic oxide powder in dry benzene was found to be 20.3 cu. cm., which was reduced to 6.5 cu. cm. by an initial concentration of 0.00827 moles of oleic acid per kg. of benzene. The presence of 0.05% of water in the titanic oxide increased this volume of the suspension to 20.8 cu. cm. or slightly more than the volume when no oleic was present. Thus water partly replaces the oleic acid in the adsorbed film at the solid-liquid interface.

4. An adsorption method for the determination of the area of the surface of a fine powder is described. The powder is immersed in extremely dry benzene to which small varying amounts of oleic acid are added. The maximum adsorption is found to correspond to a monomolecular film of the acid. It is assumed that the area occupied per molecule of oleic acid in this saturated film is the same as in a condensed film of oleic acid on water, that is 20 sq. Å., or 20×10^{-16} sq. cm. The area thus determined has been found to agree as well as could be expected with the results of a microscopic determination.

5. It is shown that the extent of the flocculation and of the settling is not, as is sometimes supposed, a simple function of the "wettability" of the powder by the liquid. Thus titanium oxide exhibits a high energy of immersion with

both oleic acid and water and a low energy with benzene, yet oleic acid in benzene deflocculates the suspension, while if water is added, the suspension becomes re-flocculated.

6. The composition of the organic liquid in which the powder is suspended has a remarkable influence upon the rate of settling of the suspension. In general the titanic oxide settles most in the end when it settles least rapidly initially.

7. In general the volume of the suspension, when it settles without vibration, varies inversely with the extent of the flocculation.

*University of Chicago,
July 27, 1931.*

MONOMOLECULAR FILMS.* THE LIQUID-LIQUID INTERFACE AND THE STABILITY OF EMULSIONS¹

EARL K. FISCHER² AND WILLIAM D. HARKINS

I. Introduction

Membranes play an extremely important part in both animal and plant physiology and in all phenomena characteristic of life. It is the purpose of the series of researches, of which this is the first, to determine something more than is now known concerning membranes, particularly of the organic type.

From the physical standpoint a membrane is a thin layer which has commonly two surfaces, or in more general terms, two interfaces between the adjacent phases. These may act to adsorb films. The layer often has the structure of a gel and is sometimes considered to be built up from micellae, which are supposed to adsorb various substances upon their surfaces.

The nature of the interfacial film in oil-in-water emulsions has been the subject of considerable theoretical speculation, since such emulsions offer, through their relatively simple constitution, a means for the determination of the thickness of interfacial films, which cannot at present be determined directly for the films present in such complicated systems as those in which membranes exist. Harkins and Beeman³ point out that in this connection several topics deserve a much more thorough investigation. These are: (1) the number of molecules of emulsifying agent per unit area of the oil-water interface, (2) the stability of emulsions and the effect of aging, and (3) the oriented wedge theory of emulsions.

Therefore in the study of membranes it seems to be of fundamental importance to determine the thickness of adsorbed interfacial films. According to McBain⁴ substances soluble in one of the phases adjacent to the interface give polymolecular films. While this is on the whole contrary to the prevalent opinion, it seemed essential to determine by as direct a method as possible what the thickness of the film actually is. It was therefore decided to investigate still further the thickness of the soap film between the phases oil and water, such as are present in an emulsion.

The first somewhat direct values for the thickness of such a soap film were obtained by Griffin.⁵ Kerosene was emulsified in aqueous soap solutions and

* Contribution from the George Herbert Jones Chemical Laboratory, University of Chicago.

¹ The work reported in this article was carried out with the aid of a fellowship from the Julius Stieglitz Fund for Research in Chemistry Applied to Medicine, established at the University of Chicago by the Chemical Foundation. The objective of the present work is a study of membrane formation.

² Julius Stieglitz Fellow, University of Chicago.

³ J. Am. Chem. Soc., **51**, 1674 (1929).

⁴ McBain and Davies: J. Am. Chem. Soc., **49**, 2230 (1927); McBain and Dubois: **51**, 3534 (1929).

⁵ J. Am. Chem. Soc., **45**, 1648 (1923).

the apparent area per molecule was calculated from the interfacial area and the amount of soap adsorbed as determined analytically. The area for the sodium oleate molecule was determined as 48 sq. Å. This is considerably higher than the area for the fatty acid in a condensed monomolecular film, ca. 20 sq. Å.⁶ At about the same time van der Meulen and Rieman⁷ studied emulsions of oil stabilized with potassium chaulmoograte and found areas for the soap molecule which corresponded with a monomolecular film within the limits of experimental error.

In the work of Griffin and that of van der Meulen and Rieman the calculation of the interfacial area was based upon the measurement of such a small number of drops that no conclusive evidence as to the molecular area was obtained. A marked improvement in the method was introduced by Harkins and Beeman, who made careful determinations, involving thousands of sizes of oil droplets, of the distribution of sizes of the oil particles in the emulsion.

In the present paper are reported results obtained by a direct microscopic count of the size distribution of oil particles to determine the specific interfacial area together with analyses to determine the amount of emulsifying agent removed. Data are also given which indicate that the soap film is labile and changes with aging of the emulsion. The results indicate that a monomolecular layer of soap molecules forms at the interface.

II. Experimental Methods

1. *Projection Equipment.*—The microscopic equipment consisted of a microscope fitted with a 61 X, 3 mm. oil immersion apochromatic objective, N. A. 1.40; a Zeiss "Homal" projection lens; a 2 inch glass prism; and a 12 mm. aplanatic condenser. The microscope was mounted on a steel base in conjunction with a clock feed arc lamp operating at 5 amperes on 110 volts direct current. A water cell was used to filter the light.

The screen, size 4 feet square, was painted with a flat white titanium oxide paint. Calibration of the optical equipment was made with an ocular micrometer slide. Magnification was such that an image of 8 mm. diameter on the screen corresponded to a particle 1 micron in diameter.

The cell was designed earlier by Harkins to reduce the tendency of the particles to segregate according to sizes. A microscope slide of thin glass contained a cell 4 mm. in diameter, the base of which is divided into minute squares, and with a depth of 0.010 mm. The cell was surrounded by a circular depression 0.5 mm. deep and 3 mm. wide. Two small grooves leading from this depression allowed any excess solution to flow off when the cover glass was put in place.

2. *Materials.* Oils.—Most of the emulsions studied were prepared from "Finol," a highly refined paraffin oil sold by the Standard Oil Co. of Indians. "Stanolax," a similar oil, but of higher viscosity, was also used.

⁶ Adam: Proc. Roy. Soc., 101A, 516 (1922).

⁷ J. Am. Chem. Soc., 46, 876 (1924).

The specific gravity of Finol is 0.885 and of Stanolax, 0.889 at 15.6°C. The viscosities of the oils are as follows: Finol, 0.170 poise; Stanolax, 0.524 poise at 37.8°C.

Soap.—Sodium oleate was prepared from oleic acid from two sources. In one series of preparations, U. S. P. oleic acid was purified through the lead and barium salt treatment⁸; in a later series Eimer and Amend C. P. oleic acid was used directly. The soaps were prepared from sodium ethoxide and oleic acid as described by Harkins and Beeman, except that from three to four recrystallizations from alcohol were made before the soap was used.

3. *Preparation of the Emulsions.*—A fresh soap solution was stirred with the oil by means of an Arnold drink mixer or a motor-driven egg beater. For emulsions prepared from comparatively dilute solutions, it was found advisable to place the soap solution in the stirring cup and to add the oil in a thin stream while stirring. The time of stirring was usually ten minutes. When a large quantity of a given emulsion was prepared it was stirred in small batches; these were combined in a large beaker, and the whole was then beaten with the motor-driven egg-beater. The resulting emulsion was placed in separatory funnels and the stoppers were sealed with paraffin. For the analyses it was convenient to have about 100 cc. of the aqueous layer. Accordingly most emulsions were made with 150–200 cc. of the oil and soap solution in equal proportions.

4. *Sampling and Measuring the Particle Sizes.*—After the emulsion had stood for several hours a partial separation of the emulsified oil and the excess soap solution took place. The lower aqueous layer of soap solution was drawn off, leaving the cream in the separatory funnel. This layer of emulsified oil retained from 25 to 40% of the aqueous solution depending on the time the emulsion had stood and the relative sizes of the particles. Emulsions with large oil particles cream rapidly. The emulsion was mixed by rotating the separatory funnel, avoiding any violent agitation. A portion of this thoroughly mixed cream was removed with a pipette and diluted approximately 1000 times with a dilute soap solution. Water was not used for the dilution, for that tended to dissolve the stabilizing soap layer and the emulsion broke while it was on the microscope slide.

A drop of the diluted emulsion was placed in the cell and covered with a thin cover glass. Finol was used for contact between the oil immersion objective and the cover glass. Measurements were made on every drop in the field. The slide was shifted by means of the mechanical stage so that a succession of independent areas were examined. The sizes were recorded with a typewriter or by an assistant who tallied the count. Four to five separate dilutions were made in each experiment. Measurements on moving spheres are difficult to make, for the size appears to change with the focus of the microscope, position of the arc carbons, and fatigue of the eyes.

5. *Analyses.*—The soap solutions were analysed as follows: A portion was pipetted into a separatory funnel, and N sulfuric acid was added in quan-

⁸ Lawrence. "Soap Films," 134 (1929); Lewkowitsch. "Chemical Technology and Analysis of Oils, Fats, and Waxes," 1, 140 (1909).

tity sufficient to provide an excess of acid over that necessary to neutralize the free alkali and to liberate the fatty acid from the soap. The aqueous mixture was extracted five times with C. P. ether, and the combined ether extracts were washed three to four times with distilled water until the wash water was neutral to litmus. The ether was evaporated nearly to the appearance of the oily fatty acid, neutral alcohol was added, and the oleic acid was titrated with standard alkali from a weight burette, using phenolphthalein as indicator. The original soap preparation was analysed at the same time that the analysis was made on the soap which had separated from the emulsion. The difference between the two titrations represents the amount of soap in the interface.

Sodium was determined both as the sulfate⁹ and as the complex uranyl-zinc-acetate.¹⁰

III. Results and Discussion

1. *Calculation of the Interfacial Area.*—The diameter and number of oil spheres give a basis for the calculation of the total area and volume represented by each size group. From these totals the specific interfacial area, or the area of interface in cm.² per cc. of emulsion is given by the following relation,

$$\text{Specific Interfacial Area (cm.}^2 \text{ per cc.)} = \frac{\text{Total Area (cm.}^2\text{)}}{\text{Total Volume (cc.)}}$$

The analytical data give the number of moles of soap removed during emulsification, calculated both from the sodium and the oleic acid determinations. The apparent area of the soap molecule, if the ratio of oil to soap solution in the emulsion is 1:1, is given by

$$\frac{\text{Area of Soap Molecule}}{\text{Molecule}} = \frac{\text{Specific Interfacial Area}}{\text{Number of Moles of Soap removed per Cu. Cm.} \times N}$$

Where N is the Avogadro constant, 6.062×10^{23} .

The number of moles of soap removed is determined in the aqueous phase, and what is desired is the number of molecules calculated as if removed from the oil phase, so in general the extra factor V_o/V_a , or ratio of volume of oil to aqueous phase is used on the right side of the equation.

2. *Particle Size Distribution.*—In the determination of the distribution of particle size in each emulsion at least 1000 droplets, and commonly from 2000 to 3000, were measured. Typical curves which show the relation of the diameter of the particles to the percentage number of particles which have that diameter, are shown in Fig. 1. In most of the emulsions of a paraffin oil the maximum number of particles corresponds to a diameter of about 1 micron. With benzene the maximum occurs at a smaller diameter.

In Fig. 2 the area per cc. is plotted against particle size. It will be observed that the greatest contribution to the area is given by particles between 6 and 7 microns in the Finol emulsions and 1 and 2 microns in the benzene emulsions. Table I is a total of a large number of experiments on emulsions prepared with Finol, but disregarding the soap concentration.

⁹ Scott: "Standard Methods of Chemical Analysis," 1, 409 (1925).

¹⁰ Barber and Kolthoff: J. Am. Chem. Soc., 50, 1625 (1928).

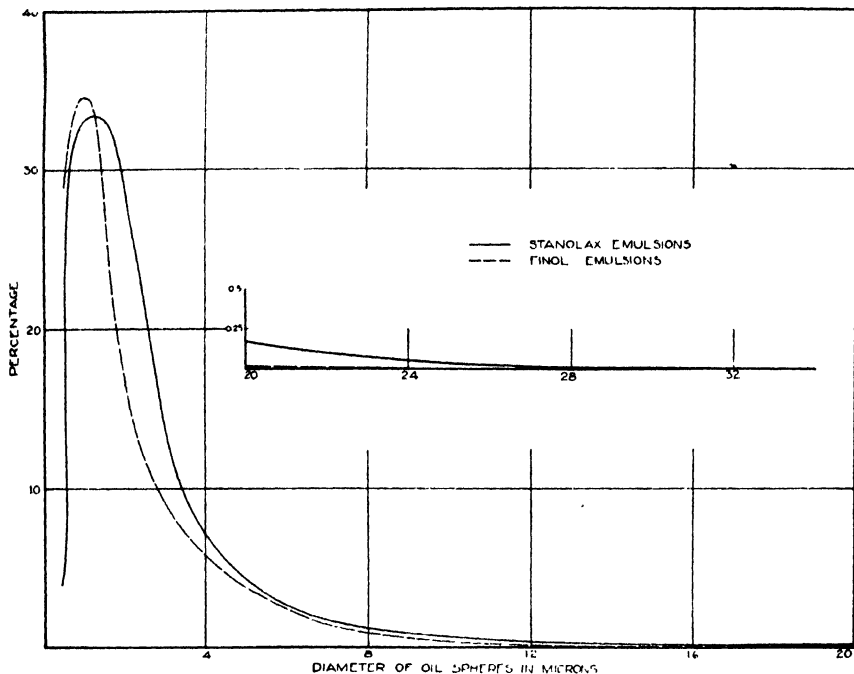


FIG. 1

Size Distribution of Oil Particles in Finol (50,000 droplets) and Stanolax Emulsions

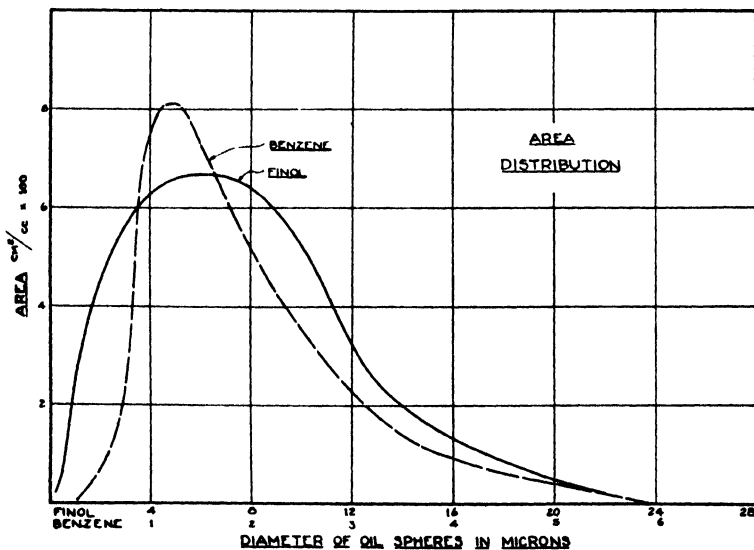


FIG. 2

Distribution of Area with Diameter in Emulsions of a Light Paraffin Oil (50,000 droplets), and of Benzene, in Water

TABLE I
Combined Size Distribution Determinations of Finol Emulsions

Size in mi- crons	Number counted	%	Number from smooth curve	%	Area cm. ² × 10 ⁵	Volume cc. × 10 ¹⁰	Area per unit- volume cm. ² /cc. × 10 ⁻¹
0.75	7518	15.204	7518	15.204	13.3	16	5.85
1	19511	39.459	19511	39.459	61.2	102	26.93
2	8259	16.703	8259	16.703	104.0	379	45.74
3	4897	9.904	4588	9.279	129.8	646	57.07
4	2569	5.195	2876	5.818	144.6	963	63.59
5	1981	4.006	1915	3.873	150.0	1254	65.94
6	1193	2.413	1352	2.734	152.4	1524	67.00
7	995	2.012	996	2.014	153.3	1792	67.42
8	828	1.675	730	1.476	146.7	1956	64.50
9	442	0.895	530	1.072	134.6	2023	59.17
10	534	1.080	383	0.775	120.2	2005	52.86
11	145	0.293	265	0.536	100.7	1846	44.26
12	144	0.291	160	0.324	72.3	1447	31.79
13	106	0.214	106	0.214	56.2	1219	24.74
14	130	0.264	78	0.158	48.0	1120	21.12
15	66	0.133	60	0.121	42.4	1060	18.64
16	52	0.105	44	0.089	35.3	943	15.55
17	26	0.054	30	0.061	27.2	771	11.97
18	21	0.042	20	0.040	20.3	610	8.95
19	7	0.014	12	0.024	13.6	430	5.98
20	19	0.038	6	0.012	7.5	251	3.31
21	0	0.000	3.5	0.007	4.7	169	2.08
22	2	0.004	2.0	0.004	3.0	111	1.33
23	0	0.000	1.1	0.002	1.8	70	0.80
24	1	0.002	0.4	0.001	0.7	28	0.31
49,446		100.000	49,446.0	100.000	1,743.8	22,735	766.90

Specific Area (per cu. cm. oil) = 7670 sq. cm.

For the purpose of this work fairly stable emulsions were required. Those prepared with soap solutions of a concentration less than 0.006 M were found unsuitable, for on standing the oil gradually separated as a clear layer above the cream. It should be remarked, however, that the initial soap concentration gives very little indication of the lower limit of the soap concentration which is necessary for a stable emulsion. The amount of soap removed is dependent on the interfacial area, in consequence of which the final soap concentration is evidently of much greater significance.

Sodium hydroxide was added to most of the emulsions to repress the hydrolysis of the soap with a concomitant liberation of the fatty acid, which is soluble in the oil phase. The effect of excess alkali on the particle size distribution is very slight.

3. *Area Occupied by Sodium Oleate Molecule.*—The experimental methods employed in this investigation were essentially statistical and subject to several errors incident to such methods. It was found possible, however, to check the calculated interfacial area within 5% when disturbing factors did not enter.

This is of about the order of accuracy usually obtained in experiments on insoluble films with the film balance.

The area per molecule of soap in the interfacial film is shown by column 7 of Table II, to lie in general between 24 and 38 sq. Å. In the experiments the *final* concentration of the soap in the aqueous phase, at the time of the separation of the emulsion, varied from 0.0025 to 0.112 moles per liter. Since, as has already been stated, the area per molecule in a condensed monomolecular film of oleic acid at zero compression is about 20.5 sq. Å., it is seen that these areas correspond to somewhat expanded monomolecular film of oleic acid. However, the presence of electrostatically bound sodium ions instead of the more closely bound hydrogen of the acid, undoubtedly has an effect on the area, but does not change the monomolecular character of the film. More concerning this effect will be presented in the next section.

TABLE II

Molecular Areas for the Soap at the Interfacial Film in Oil-in-Water Emulsions

Emulsion Number	Age of Emulsion in hours	Specific Interfacial Area $\text{Cm}^2 \text{ per cc.} \times 10^{-3}$	Molarity of Equilibrium Soap	Molarity of Initial Soap	Normality of Sodium Hydroxide	Area of Sodium Oleate Molecule from Oleic Acid Analysis Sq. Å	Area of Sodium Oleate Molecule from Sodium Analysis Sq. Å
47				0.004	0.0004	Emulsion Unstable	
48	18	11.2	0.0025	0.0066	0.0002	44.5	44.6
33 ¹	138	6.08	0.0043	0.0100	0.004	26.1	
46 ²	78	11.9	0.0047	0.0083	0.0002	18.2	
34 ⁶	720	5.58	0.0053	0.0114	0.004	30.2	29.6
32	168	6.00	0.0059	0.0100	0.004	24.2	26.1
39	3 ³	5.43	0.00718	0.01040	0.002	27.8	28.9
40	48	6.64	0.00634	0.01030	0.002	27.6	29.6
43 ⁴	144	11.2	0.00920	0.01800	0.0018	38.2	32.5
36 ⁵	190	6.17	0.0199	0.0254	0.0024	37.4	46.0
23	72	9.96	0.0341	0.0390	0.0013	33.6	38.8
29	36	10.8	0.0655	0.0710	0.025	32.4	25.2
42	3 ³	12.9	0.112	0.120	0.020	27.0	26.4

¹ Ratio of oil to soap solution, 3:2.

² Ratio of oil to soap solution, 1:3.

³ Separated by centrifuging three hours.

⁴ Ratio of oil to soap solution, 1.82:1.

⁵ Ratio of oil to soap solution, 2:1.

The molecular area for sodium oleate in two emulsions, to which no sodium hydroxide or other base had been added, of ages 3 and 120 hours, is given as 29 and 27 sq. Å. in column 7 of Table III. The apparent areas in column 6 are listed only for comparison. The difference between the values in the two columns represents oleic acid which has been produced by hydrolysis and which is dissolved in the oil droplets, but is in the calculation considered as in the film. The value 11.7 for emulsion 50D would give the idea that the film is two molecules thick to anyone who does not take the hydrolysis into account. However, results obtained with entirely similar emulsions indicate that it is monomolecular.

TABLE III

Molecular Areas for the Soap at the Interfacial Film in Oil-in-Water Emulsions. (No excess Alkali present in Soap Solutions)

Emulsion Number	Age of Emulsion in Hours	Specific Interfacial Area, Cm^2 per cc. $\times 10^{-3}$	Molarity of Equilibrium Soap	Molarity of Initial Soap	Apparent ² Area of Sodium Oleate Molecule from Oleic Acid Analysis Sq. Å	Area of Sodium Oleate Molecule from Sodium Analysis Sq. Å
37	3 ¹	7.23	0.0102	0.0154	23.0	29.0
50A	120	8.30	0.0125	0.0204	17.3	27.0
50D	1460	6.28	0.0114	0.0204	11.7	

¹ Separated by centrifuging three hours.

² On account of hydrolysis the values listed in this column are entirely fictitious, since they have been calculated by including oleic acid dissolved in the oil, as well as that in the film. The molecular areas are given in the last column.

4. *Decrease in Molecular Area with the Age of the Emulsion.*—If oil is emulsified in a *dilute* soap solution it is often found to exhibit a somewhat **large** molecular area, of the order of 40 to 50 sq. Å., for the soap in the interfacial film.

In such cases it was discovered by Harkins and Beeman that the mean size of the oil droplets in the emulsion increases with its age. Their data indicate that concomitantly with this decrease of size and the corresponding decrease in interfacial area, *there is a decrease in the molecular area of the soap*. However, they did not determine the extent of this decrease, although they developed an hypothesis, given later, concerning it.

In order to determine the decrease in molecular area, the writers have carried out a very carefully conducted experiment on the aging of an emulsion produced by 0.02 molar sodium oleate as an emulsifying agent, in the presence of 0.004 molar sodium hydroxide, used to repress hydrolysis.

Fig. 3 shows that the specific interfacial area for this emulsion falls rapidly as follows, where the values are given in 10^3 sq. cm.: 9 at the end of 3 hours, 7 at 18 hours, 6 at 50 hours, 5.5 at 648 hours, and 5.25 at 2668 hours (111 days).

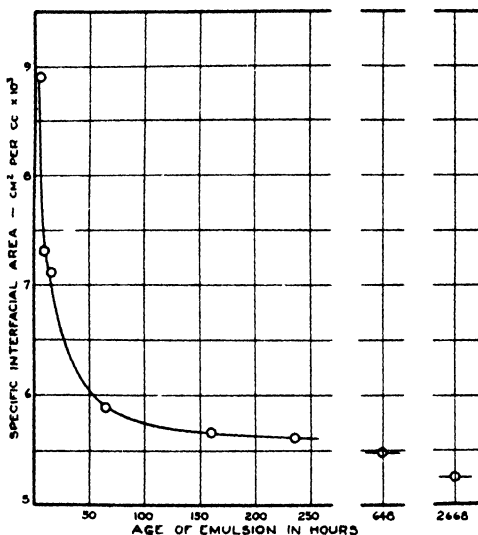


FIG. 3

Decrease in Interfacial Area with the Age of an Emulsion in which the Emulsifying Agent was 0.02 molar Sodium Oleate

This reduction of area is not brought about by any separation of the oil in bulk, but by a disappearance of small droplets by coalescence with larger ones, and the resultant growth of the larger drops. The change in the distribution of sizes is given in Fig. 4, which represents a period of 3 months. Many more curves were determined, but their inclusion in a single figure makes it difficult to follow. At the end of 5 hours after the preparation the peak in the curve is

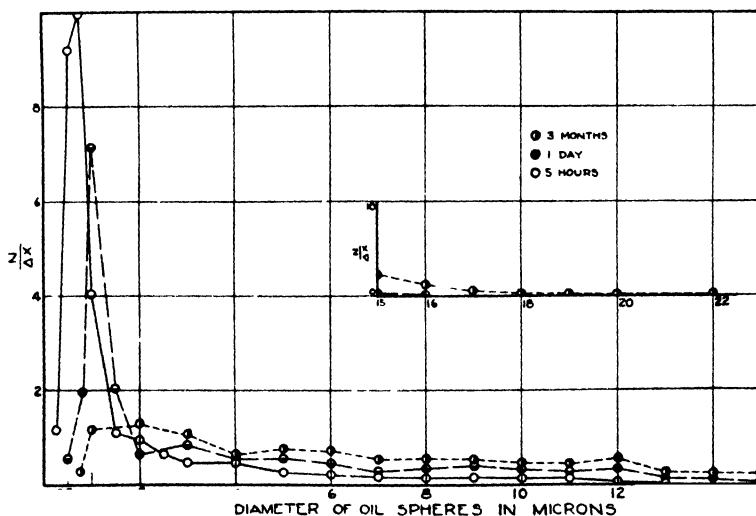


FIG. 4

Change in the Distribution of Diameters in an Emulsion with Its Age

at a diameter of 0.75 micron, and a height 10, at the end of 1 day at 1 micron and a height 7.2, and at the end of 3 months the curve is greatly flattened, with the maximum number of particles at 1 and 2 microns, and a maximum height of 1.2. The largest drops at 5 hours have a diameter of 13, at 1 day 16 and at 3 months 22 microns. The other six curves, which have not been included, illustrate very beautifully the gradual decrease in the height of the peak, its shift to the right, and the flattening of the curve. Similar curves, with a more rapid change were obtained by the use of more dilute soaps in the work of Harkins and Beeman.

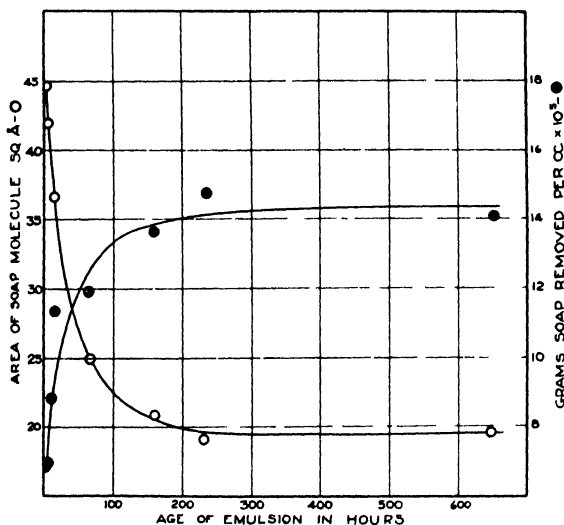


FIG. 5

[Decrease of the Molecular Area of Sodium Oleate in Emulsion with Its Age

A large amount of this emulsion was prepared, and stored in a considerable number of large separating funnels. The first sample used for a determination of the distribution of sizes and for analysis, was centrifuged at the end of 3 hours. The other samples were allowed to cream under the influence of gravitation. The samples which were kept for longer periods than the time necessary to separate the emulsion in this way, were mixed with extreme gentleness by rolling slowly by hand, in such a way as to prevent further emulsification. The mixing was essential to allow the film to come more closely into equilibrium with the aqueous phase.

The values listed in column 4 of Table IV show how the concentration of the aqueous soap solution decreases with time, to the extent of 14% in 10 days, and not at all in the succeeding 17 days.

The initial area per molecule of soap is 44.6 sq. Å. (Fig. 5 and Table IV). A decrease of 14% in the soap content of the aqueous phase does not add sufficient soap to the film to change this highly expanded to a condensed monomolecular film, in which this area is reduced to about 20 sq. Å. The pro-

duction of a condensed film is thus due to two factors: 1. A reduction in the soap concentration of the aqueous phase. 2. A decrease in the interfacial area.

The first of these is represented in Fig. 5 by the amount of soap removed per cu. cm. This amount increases for 10 days and then remains practically constant.

The decrease in the molecular area of the soap with the age of the emulsion is given in column 5 of Table 4 and in Fig. 5. The area decreases from 44.6 sq. Å. at the end of 3 hours, along a quite smooth curve to a constant minimum of about 19.6 sq. Å. at the end of 10 days. At the end of 27 days the area was the same, within the limits of accuracy.

TABLE IV

Effect of Age of Emulsion on the Calculated Molecular Area for the Soap at the Interfacial Film in Oil-in-Water Emulsions

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Emulsion Number	Age of Emulsion in Hours	Specific Interfacial Area $\text{Cm}^2 \text{ per cc.} \times 10^{-3}$	Molarity of Equilibrium Soap	Area of Sodium Oleate Molecule from Oleic Acid Analysis Sq. Å.	Area of Sodium Oleate Molecule from Sodium Analysis, Unanlyl-Zinc-Acetate Method. Sq. Å.	Area of Sodium Oleate Molecule from Sodium Analysis Sodium Sulfate Method. Sq. Å.
54	3	6.08	0.01776	44.6		41.5
53	5	5.79	0.01772	41.9		31.5
(52)	10	7.31	(0.03708)	(41.3)	(41.0)	
51A	16	7.12	0.01628	31.6	25.8	27.7
51B	64	5.88	0.01611	24.9		
51C	160	5.66	0.01553	20.9		19.2
51D	236	5.61	0.01515	19.1	19.8	
51E	648	5.48	0.01538	19.6		

Initial concentration of sodium oleate, 0.02000 M., in all emulsions except No. 52 which was 0.04000 M.

Initial concentration of excess sodium hydroxide, 0.0040 N.

Thus this series of experiments confirms the point of view of Harkins and Beeman, when they say, concerning an emulsion in which there was at first a highly expanded monomolecular film: "Such an emulsion is not stable, and on standing the area of its interface decreases, and the area per molecule of soap in the film decreases, until the area becomes about that for a tightly packed (or condensed) monomolecular film."

In some cases, however, emulsions produced by sufficiently concentrated solutions of emulsifying agents, 0.1 molar soap or more concentrated, do not exhibit any considerable decrease of interfacial area with time: indeed in some instances emulsions have stood for years without any detectable change. For example two emulsions in which 0.1 molar sodium oleate was the emulsifying agent as determined by them in one of these emulsions, and that determined

by Miss Sophie Berkman four years later were in as close agreement as if the determinations had been made on two samples of the same emulsion by the same worker at the same time. Thus if the interfacial soap film is initially a condensed monomolecular film, the tendency for the drops to grow in size by coalescence seems to be largely, and in some cases practically wholly, removed.

5. *Additional Conclusions.*—The work already described proves definitely that: (1) the interfacial film which produces emulsification is not colloidal, and (2) it is not thicker than one molecule of the emulsifying agent, when the emulsifying agent is molecularly dispersed in its solution.

6. Both the aqueous phase of the emulsions, after the separation of the cream, and the soap solutions from which the emulsions were prepared, have been investigated by the use of a Leitz slit ultra-microscope. It seems probable from this work that the ultramicroscopic droplets of oil do not add appreciably to the total area of the oil-water interface.

A portion of the emulsion was centrifuged for five hours at high speed. The oil particles separated as a thick cream, leaving a slightly opalescent solution which contained few particles visible in the microscope. This aqueous layer was diluted and examined in the slit ultramicroscope. The dilution was such that from two to five particles were present in the field at a given instant. The colloidal particles present in the original soap solution were counted in the same way and subtracted from the count which was made on the emulsion. Appropriate corrections were made for the decrease in the soap concentration during emulsification. The volume of oil in the emulsions was determined by a modified Babcock test. From these data the average particle diameter was calculated to be 0.22 micron. Although this method at best gives only an approximate *average size*, the conclusion seems justified that the ultra-microscopic oil particles do not contribute largely to the area of the interface. Any area which is of this type, of which account has not been taken in this work, would slightly increase the molecular areas given in the paper, and thus would increase the evidence that the films are truly monomolecular.

IV. Summary

1. One of the most important results of the work presented in this paper is to give definite evidence which shows the validity of the hypothesis presented earlier by Harkins and Beeman, that the stability of an emulsion increases in general as the emulsifying film changes from an expanded to a condensed monomolecular film. Thus they say concerning an emulsion produced by a very dilute emulsifying agent: "Such an emulsion is not stable, and on standing the area of its interface decreases, and the area per molecule of soap in the film decreases, until the area becomes about that for a tightly packed monomolecular film." It is shown that in addition, additional emulsifying agent is withdrawn from the solution and goes into the film, as the emulsion ages. Thus in an emulsion produced by 0.02 molar soap the initial molecular area of soap in the film was 44.5 sq. Å., and this decreases gradually along a smooth curve with respect to time, until the area falls to about 20 sq. Å., at which value it remains constant.

2. Certain emulsions produced by sufficiently concentrated soaps as emulsifying agents very rapidly produce a condensed monomolecular film at the interface. Such emulsions are often stable over a period of years, without any appreciable decrease.

3. A distribution curve is presented for the variation of the interfacial area with the diameter in emulsions of a paraffin oil in water, produced by sodium oleate as an emulsifying agent. The curve gives the results of the measurement of about 5×10^4 droplets. A similar curve for benzene, obtained from the measurement of a smaller number of droplets is given for comparison. The maximum area occurs at 6.3 microns diameter with the light paraffin oil, and at about 1.2 microns with benzene.

4. The most important result of this work is that it definitely contradicts the idea that surface and interfacial films are several molecules thick in all cases in which the adsorbed substance is soluble in one of the phases. Soap films in particular have been recently supposed to be polymolecular, but it is now shown by accurate work, that they are monomolecular, and either expanded or condensed.

The writers wish to thank Julius Stieglitz and the Chemical Foundation for assistance which has made this investigation possible.

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University of Chicago.*

ELECTROKINETIC POTENTIALS. X. THE EFFECT OF PARTICLE SIZE ON THE POTENTIAL*

BY HENRY B. BULL AND ROSS AIKEN GORTNER

Introduction

The present study was an outgrowth of an effort to obtain a standard material for the investigation of electrokinetic phenomena. Much of the work on electrokinetics has been done on ill-defined materials and it has been almost impossible for other workers to repeat each other's results with any degree of precision. It seemed desirable that the different methods for determining the electrokinetic potentials be checked against each other using the same standard material. Accordingly it was decided to use very pure quartz powder for this purpose. Investigation however showed that the situation was not simple because the electrokinetic potential on quartz particles depends upon particle size. Accordingly it was necessary to study the effect of particle size on the potential.

Mooney¹ reports a decrease in cataphoretic mobility of red oil, benzyl chloride, iodobenzene, tribromhydrine and dimethylaniline measured in distilled water as the particle size is decreased. He found the mobility to start decreasing with the decreasing particle size at about 150μ .

Abramson's and L. Michaelis'² work on the cataphoresis of protein-covered particles seems to indicate that with such particles the mobility is independent of the size.

Experimental

Five pounds of pure well-formed quartz crystals were ground[†] to pass a twenty-mesh sieve, digested with aqua regia for six hours, washed by decantation forty times with distilled water and ten times with conductivity water, sucked dry on a Büchner funnel, and then heated at 800°C . for 12 hours in a muffle furnace. This quartz was subsequently separated into nine portions by means of different mesh screens.

The only electrolyte solutions used in this investigation were 1.0×10^{-4} N and 2.0×10^{-4} N NaCl. The NaCl was the purest obtainable. It was dried at 400° before weighing for solution. The water used in making the solution was twice distilled and had a conductance of about 1.5×10^{-5} mhos. The volumetric apparatus was calibrated. The solutions were used the same day they were made.

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¹ J. Phys. Chem., 35, 331 (1931); Phys. Rev., (2) 23, 396 (1924).

² J. Gen. Physiol., 12, 587 (1929).

[†] Thanks are due Dr. C. C. Furnas of the Experimental Station, U. S. Bureau of Mines for grinding this quartz.

The streaming potential method was used for determining the electrokinetic potential on the quartz. The apparatus was the same as that described by Bull and Gortner.³ No constant temperature bath was employed but all the work was done in a room the temperature of which was $24.5 \pm 0.5^\circ$.

The conductivity of the salt solution in contact with 4.6μ size quartz was determined in the streaming potential cell. The other conductivities reported are those of the solution in bulk. This is deemed permissible because it was found that the surface conductance of the quartz larger than 4.6μ was small enough so that surface conductance could be neglected. The bulk conductivi-

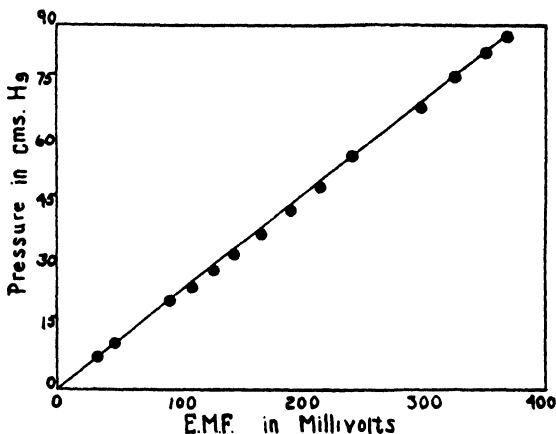


FIG. 1

Showing the relation between the streaming potential and the pressure, as experimentally determined in our apparatus using a cellulose diaphragm and 1.0×10^{-4} N NaCl, as the liquid was being streamed thru the diaphragm

ties were determined using a conductivity cell described in detail by Washburn⁴ for specific conductances in the range between 10^{-6} and 10^{-4} mhos. The resistance was determined with a Leeds and Northrup alternating current galvanometer. The cell constant was determined with both N/10 and N/100 KCl using the Kahlrausch values. Head phones tuned to 1000 cycles vibrations were used to determine the cell constant.

Since it was our original intention to obtain a standard material for electrokinetic work, and to check the streaming potential method against cataphoretic methods, using this material, we ground some of our quartz in an agate mortar until it remained in suspension when mixed with water. This quartz was packed in a diaphragm and its ζ -potential determined. The theory demands that there exist a linear relationship between the pressure applied on the liquid streaming thru the diaphragm and the electrical potential observed across the diaphragm as is shown in Fig. 1 for cellulose and an aqueous solution of 1.0×10^{-4} N NaCl.

³ J. Phys. Chem., **35**, 309 (1931).

⁴ J. Am. Chem. Soc., **38**, 2431 (1916).

To our surprise we found no such relationship with our quartz but instead, as is shown in Table I and Fig. 2, where the pressure is the abscissa and the streaming potential is the ordinate, we found a decreasing slope as we increased the pressure.

We found the same behavior to hold with 2.0×10^{-4} N NaCl and in later work used this concentration. Much time was spent in trying to determine the cause of this deviation from a straight line.

It is one of the fundamental requirements in the derivation of the streaming potential equation for calculating the zeta potential that Poiseuille's law for the flow of liquids thru capillaries be obeyed. It occurred to us that our difficulty might lie in the failure of this law of flow at higher pressures. This law states that the volume of liquid passing any cross section of a capillary in unit time is

$$V = \frac{\pi r^4}{8\eta} \frac{P_1 - P_2}{l}$$

where

η = coefficient of viscosity of the liquid flowing

r = radius of capillary

$P_1 - P_2$ = difference in pressure between the two ends of the capillary

l = length of capillary.

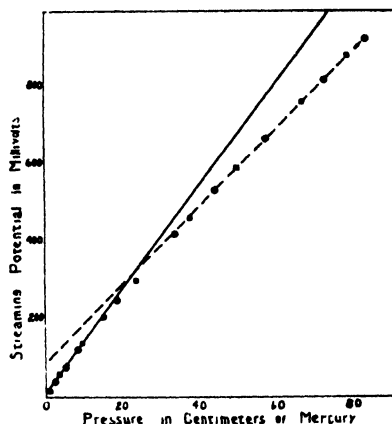


FIG. 2

Showing relation between pressure and the streaming potential with a heterogeneous mixture of quartz particles (sizes between $.5\mu$ and 36μ) and 1.0×10^{-4} N NaCl

TABLE I

Showing the Relationship between the Streaming Potential and Hydrostatic Pressure for a Quartz Diaphragm through which a 1.0×10^{-4} N Solution of NaCl was being streamed, the Quartz Particles being Non-uniform in Size.

Pressure (P) cm. Hg	Streaming Potential (H) mv.	H/P	Pressure (P) cm. Hg	Streaming Potential (H) mv.	H/P
With decreasing pressure					
1.0	19.5	19.50	77.5	885.0	11.42
2.4	40.5	16.88	66.1	767.5	11.61
5.1	77.5	15.20	49.7	593.0	11.93
8.7	124.0	14.25	37.2	463.0	12.45
18.8	251.0	13.35	23.5	304.0	12.94
33.7	423.0	12.55	15.1	208.0	13.78
43.9	536.0	12.21	9.7	141.5	14.59
57.0	670.5	11.76	5.5	86.5	15.73
71.7	821.0	11.45	3.5	61.5	17.57
82.1	928.0	11.30	1.2	25.5	21.25

One test of this law is to determine the relation between the rate of flow of a liquid thru a capillary as a function of the pressure applied. Poiseuille's law demands that this function be linear and that the volume of the liquid in unit time per unit pressure be a constant. Accordingly the rate of flow thru a quartz diaphragm at different pressures was studied. The quartz diaphragm used was prepared from the same sample of quartz as that employed to obtain the data for Table I. The results shown in Table II were obtained. The data in Table II show that there is not a sufficient deviation from Poiseuille's law to explain the lack of linearity between the pressure and the streaming potential.

TABLE II

Testing Poiseuille's Law of Flow thru a Quartz Diaphragm made of a Heterogeneous Mixture of Quartz Particles (sizes between $.5\mu$ and 36μ in diameter) with an Aqueous Solution of 2.0×10^{-4} N NaCl.

Pressure (P) cm. Hg	Rate of Flow (V) grams of solution per min	V/P	Pressure (P) cm. Hg	Rate of Flow (V) grams of solution per min	V/P
8.0	.350	.0437	58.4	2.709	.0464
17.2	.786	.0457	67.0	3.126	.0466
26.3	1.190	.0452	75.9	3.462	.0456
37.4	1.750	.0468	82.3	3.950	.0480
48.0	2.260	.0461			

It was then decided to investigate the effect of particle size on the streaming potential. To this end that portion of the quartz powder which passed a 20 mesh sieve was further separated by sieving into nine portions. The particle size was assumed to be the arithmetic mean between the sieve size it was passed thru and that upon which it was retained. The three smallest sizes, i.e., 4.59μ , 31.1μ , and 74.91μ were determined by measurement of a number (75-100) of particles with a calibrated microscope and then taking the average of these measurements.

The 4.59μ size was packed in a diaphragm 1.1 cm. long and 2 cm. in diameter. The larger sized samples were packed in diaphragms 9.5 cm. long and 2 cm. in diameter. With this arrangement a convenient rate of liquid flow was obtained. Perforated gold electrodes were used at each end of the diaphragm. With the smaller quartz (98μ and below) a thin layer (about 1 mm.) of 214μ quartz was placed at each end of the diaphragm to keep the smaller quartz from washing thru the perforations in the electrodes. The diaphragms were washed with at least 500 cc. of the solution and were then allowed to remain 12 hours in contact with a portion of the electrolyte solution to be used. This portion was replaced by fresh solution before a measurement was attempted. The values reported and used in the graph of the results are the average of at least six or more readings.

Results

The results of the electrokinetic studies on the nine different particle sizes of the quartz are given in Table III thru Table XI, summarized in Table XII and graphed in Fig. 3.

TABLE III

 2×10^{-4} N NaCl and 630μ Quartz

Pressure cm. in Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. Hg (P)	Streaming Potential in millivolts (H)	H/P
2.03	61.0	30.06	0.98	28.0	28.56
0.23	7.0	30.74	0.57	18.0	31.82
1.76	50.0	28.42	1.06	33.0	31.14
2.70	80.0	29.65	0.78	20.0	25.27
3.49	90.0	25.70	Average H/P = 29.04		

TABLE IV

 2×10^{-4} N NaCl and 330μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
0.71	20.0	28.29	1.23	35.0	28.42
1.33	40.0	30.06	0.90	25.0	27.61
.80	25.0	31.14	Average H/P = 29.10		

TABLE V

 2×10^{-4} N NaCl and 214μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
2.57	80.0	31.01	2.90	90.0	31.01
2.54	75.0	29.51	2.36	70.0	29.65
2.71	80.0	29.51	3.27	100.0	30.46
3.01	90.0	29.92	Average H/P = 30.15		

TABLE VI

 2×10^{-4} N NaCl and 163μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
2.93	90	30.76	3.31	100	30.23
3.08	90	29.21	2.81	80	28.49
3.28	95	28.97	2.84	80	28.19
Average H/P = 29.31					

TABLE VII

 2×10^{-4} NaCl and 128μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
1.11	31.0	27.92	1.29	34.0	26.36
2.72	74.0	27.21	3.57	99.0	27.73
4.61	128.0	27.77	9.9	254.0	25.66
11.4	293.0	25.70	17.6	453.0	25.74
20.9	544.0	26.03	22.9	598.0	26.11
Average H/P = 26.62					

TABLE VIII

 2×10^{-4} N NaCl and 98μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
3.86	104.0	26.94	19.5	465.0	23.85
9.1	223.0	24.51	34.2	830.0	24.27
21.9	545.0	24.89	1.61	38.0	23.60
31.4	775.0	24.68	3.57	84.0	23.53
1.53	40.0	26.14	8.6	188.0	21.86
4.08	103.0	25.25	18.7	423.0	22.62
10.6	250.0	23.58	34.0	780.0	22.94
Average H/P = 24.19					

TABLE IX

 2×10^{-4} N NaCl and 74.9μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
1.90	40.0	21.05	4.06	97.0	23.89
3.97	89.0	22.42	9.60	216.0	22.50
9.60	210.0	21.88	19.50	453.0	23.23
18.70	431.0	23.05	27.60	635.0	23.01
28.40	665.0	23.42	37.30	850.0	22.79
1.42	35.5	25.00	Average H/P = 22.93		

TABLE X
 2×10^{-4} N NaCl and 31.1μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
1.41	30.0	21.28	29.70	595.0	20.03
3.75	78.0	20.80	40.30	818.0	20.30
9.20	177.0	19.24	52.50	1097.0	20.90
19.80	392.0	19.80	52.10	1099.0	21.09
29.80	604.0	20.27	4.50	85.0	18.89
40.40	826.0	20.45	9.00	166.5	18.50
51.10	1057.0	20.68	20.10	388.0	19.30
62.80	1310.0	20.86	29.00	567.0	19.55
1.33	30.0	22.56	40.10	792.0	19.75
3.77	77.0	20.42	50.60	1006.0	19.88
9.60	181.0	18.85	59.80	1191.0	19.92
19.60	381.0	19.44	Average H/P = 20.12		

TABLE XI
 2×10^{-4} N NaCl and 4.59μ Quartz

Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P	Pressure in cm. of Hg (P)	Streaming Potential in millivolts (H)	H/P
3.47	23.0	6.63	47.20	302.5	6.41
11.60	65.0	5.60	62.50	392.0	6.27
18.40	116.0	6.30	73.50	456.0	6.20
31.40	199.0	6.34	Average H/P = 6.25		

TABLE XII

Summary of Electrokinetic Potentials at a Quartz-Aqueous 2.0×10^{-4} N NaCl Interface. Showing the Effect of Particle Size.

Particle diameter in μ	$\log \mu$	H/P	$\kappa_s \times 10^5$ mhos	$H\kappa_s/P$	$\log H\kappa_s/P$
630	2.799	29.04	28.90	83.93	1.924
330	2.519	29.10	28.90	84.10	1.925
214	2.330	30.15	28.90	87.13	1.940
163	2.212	29.31	28.90	84.71	1.928
128	2.107	26.62	28.90	76.93	1.886
98	1.991	24.19	28.90	69.91	1.845
74.9	1.875	22.93	28.90	66.27	1.821
31.1	1.493	20.12	28.90	58.15	1.765
4.59	0.662	6.25	35.81	22.38	1.350

Discussion

It is our belief that the deviation from a linear relation between pressure and the streaming potential is due to the fact that the ζ -potential on the quartz particles is different for different size particles. This lack of linearity was observed only with a heterogeneous mixture of particle sizes. When the quartz was separated into its fractions of relatively uniform particle size a

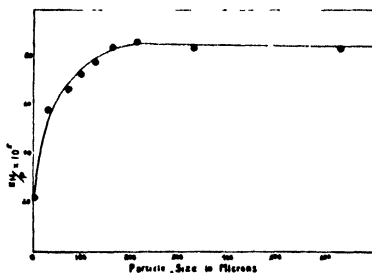


FIG. 3

Showing the relation between the electrokinetic potentials at a quartz-aqueous 2.0×10^{-4} N NaCl interface for different particle sizes

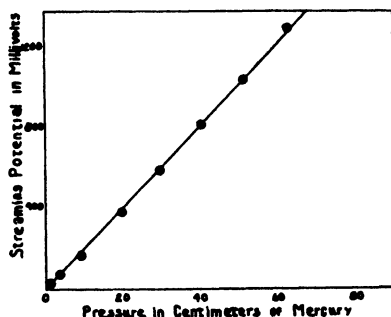


FIG. 4

Showing relation between pressure and electrokinetic potential for a homogeneous particle size. (Data from Table X. 2×10^{-4} N NaCl and 31.1μ quartz).

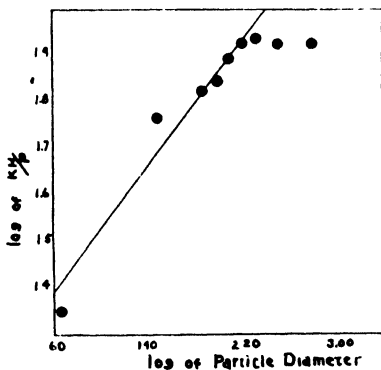


FIG. 5

Plot of $\log H_K/P$ against $\log \mu$. The straight line is drawn on the assumption that H_K/P varies as the cube root of μ

good straight line was obtained between pressure and the streaming potential as is demonstrated in Fig. 4 plotted from the data of Table X.

It seems probable that as the pressure is increased the smaller quartz particles are displaced into the places of maximum flow, thus protecting the larger particles and diminishing their importance in the picture, and, since it is the larger particles which have the higher potential, naturally the ratio between the streaming potential and pressure decreases, thus producing the effect shown in Fig. 2.

In Fig. 5 the log of the particle diameter is plotted as the abscissa against $\log H_K/P$ as the ordinate.

From a particle size of 4.59μ to 214μ an approximate straight line is obtained whose slope is $1/3$, which means that H_K/P varies roughly as the cube root of the diameter of the particle. It is suggestive that as we decrease the particle size the ratio of edge length and number of corners to the surface area of the particles increases. Now there is reason to believe that, due to un-

satisfied valencies in the crystal lattice, more adsorption occurs at the edges and corners than on the flat surfaces^b so that as we decrease the particle size of the quartz we should expect more adsorption per unit area, which would be equivalent to having a higher salt concentration at the interface which, in turn, would undoubtedly produce a lower electrokinetic potential and thus account for the fact that the smaller quartz particles were found to have a smaller electrokinetic potential.

Summary

1) Pure quartz crystals were ground to pass a 20 mesh sieve, thoroughly cleaned and separated into nine portions according to the particle size. Electrokinetic studies were conducted on this quartz in an aqueous solution of 0.20×10^{-3} N NaCl.

2) In a heterogeneous mixture of different size quartz particles, no linear relation was found between the pressure forcing the liquid thru the quartz diaphragm and the streaming potential. Poiseuille's law was found to hold with this quartz.

3) In a more homogeneous mixture of quartz particles a good linear relation was found between the pressure forcing the liquid thru the quartz diaphragm and the streaming potential.

4) Between a particle size of 4.59μ and 214μ the surface potential was found to increase roughly as the cube root of the diameter.

5) It is suggested that the lack of linearity between the streaming potential and the pressure in a heterogeneous mixture of particle sizes is due to the fact that the smaller particles have a smaller surface potential.

6) It is also suggested that the smaller particles have a smaller surface potential because they adsorb a greater amount of salt per unit area.

^b Taylor: J. Phys. Chem., 30, 145, (1926).

STREAM POTENTIAL DETERMINATIONS ON GLASS CAPILLARIES OF VARIOUS SIZES

BY H. L. WHITE, FRANK URBAN AND E. T. KRICK

An earlier report¹ stated that no stream potential could be detected (method sensitive to 0.1 mv.) across a cellophane membrane through which 0.0005 M KCl was being forced at a pressure of 8 cm. of Hg. When this solution is forced through a glass capillary at this pressure a stream potential of about 125 mv. is developed. The hypothesis was suggested that the failure of the potential to develop across the membrane was due to the discharge through relatively inactive pores of the E.M.F. set up across the active pores. This conception would explain the absence of potential regardless of the reason or reasons for the variations in the behavior of the pores. One factor affecting the magnitude of the stream potentials developed by the various pores might conceivably be pore size. Since it was impossible to investigate experimentally the individual pores in a membrane, glass capillaries were employed as pore models. Previous workers have found stream potential independent of capillary diameter, so long as Poiseuille's law held. Their "small" capillaries, however, did not differ greatly in size from their "large"; no previous workers have used capillaries near our range of small sizes. Our previously reported attempts to investigate stream potential as a function of capillary diameter were unsuccessful because we had not succeeded in establishing the conditions essential to reproducibility of results. During the past year we have succeeded, but only after many fruitless endeavors which need not be described. Most previous workers in this field have had, one would judge from their reports, but little difficulty in getting reproducible results. Our experience has more resembled that reported by Lachs and coworkers.²

For our work i.e., the comparison of the stream potentials exhibited by glass capillaries of various diameters, all other factors constant, it was necessary that a method of treating the capillaries be developed which could be depended upon to give repeatedly the same potential for all capillaries of a given diameter. This imposes much more stringent conditions of reproducibility than are demanded in work involving the comparison of various solutions passed consecutively through one and the same capillary. After trying many procedures, including various treatments with chromic acid solutions, we have adopted the following standardized technique. Water at 60-80° is first sucked through the capillary for an hour. The capillary with its glass holder is then sealed to an all-glass apparatus, as shown in Fig. 1, and steam is forced through it at a pressure of 35 cm. of Hg. The water used is double distilled, the second distillation being in a pyrex still from alkaline KMnO_4 .

¹ Bishop, Urban and White: *J. Phys. Chem.*, **35**, 137 (1931).

² Lachs and Kronman: *Bull. intern. l'acad. polonaise*, (B) 289 (1925); Lachs and Biczysk: (B) 360 (1930); *Physik. Z.*, **28**, 556 (1927); *Z. physik. Chem.*, (A) **148**, 441 (1930).

solution. The period of steaming is 60 minutes; the coil of resistance wire around the capillary is heated just red for three periods of 10 minutes each alternating with periods when the wire is not heated. The capillary with its holder is then removed and set aside in the air until the next day when the experiment is carried out. This treatment is repeated before each stream potential determination. We have found a somewhat higher potential when the determination is made immediately after the steaming process but the range of fluctuation of repeated determinations is somewhat greater than if the capillary is allowed to sit for a day. The sucking through of saturated or half saturated chromic acid solution at 90° for one hour followed by prolonged rinsing and sucking through of distilled water gives satisfactory reproducible results for capillaries of ordinary size; the stream potential of many capillaries with such treatment is about 10 per cent lower than with the steaming treatment.¹ With the smallest capillaries, however, ($4.5-15\mu$) the chromic acid treatment gave much lower values than steaming and the variation was greater. Whether or not this is due to inability to remove the last traces of chromic acid from their walls we cannot say. All solutions were made up with double distilled water and the water and solutions never allowed to touch anything but pyrex glass.

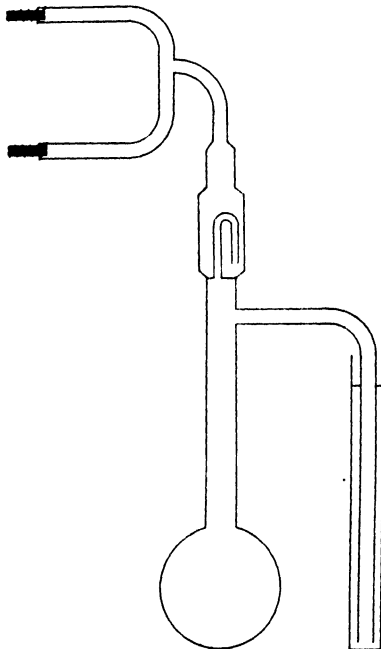


FIG. 1

Apparatus for steaming capillaries

Lachs and Biczyn² state that the CO_2 content of freshly distilled water profoundly influences the stream potential of dilute salt solutions, and aerate the solutions with untreated room air before using them. We have found that the stream potential of the relatively concentrated solution (0.0005 M KCl) used in all our experiments here reported is unchanged by prolonged aeration. We

¹This higher potential after steaming is not always exhibited. Certain capillaries have repeatedly shown the same value with the chromic acid treatment as with the steaming. In the large group of capillaries which we have used are certain exceptionally well behaved individuals which will always show essentially the same stream potential, even with slight modifications in the preliminary treatment. Even these capillaries, however, show the following differences after steaming and after chromic acid; after steaming the initial readings are higher and fall to the stable or equilibrium level in the course of 15 to 60 minutes, while after chromic acid the potential rises to the stable level. Most capillaries are extremely susceptible to slight changes in treatment. We have made so many determinations on many of our capillaries that we know the dependable and the undependable ones; it has sometimes happened that the same piece of glass tubing has furnished capillaries of both types.

² Loc. cit.

have also found that removal of possible traces of ammonia and sulphur compounds, as well as of CO_2 , from the compressed air by a train of wash bottles containing, in order, 1 per cent iodine plus 10 per cent KI, $\text{N}/5 \text{ Na}_2\text{S}_2\text{O}_3$, 50 per cent KOH, 5 per cent H_2SO_4 , and distilled water did not affect the stream potential. It eventually became evident that the fluctuations shown by a given capillary must be due to changes in its walls; these were finally minimized by the treatment described above. In all experiments the pressure applied was 60 cm. of Hg.

The arrangement for measuring potentials was essentially that described in an earlier communication, i.e., the balancing of the stream potential and potential led off from a L. & N. type K potentiometer across a 2 mf. condenser, which was discharged through a 10^{-10} amp. sensitive galvanometer. The leads from the capillary were through $\text{N}/10$ KCl calomel electrodes; possibility of diffusion of KCl from the electrodes into the capillary has been certainly excluded. All units of apparatus were mounted separately on one fourth

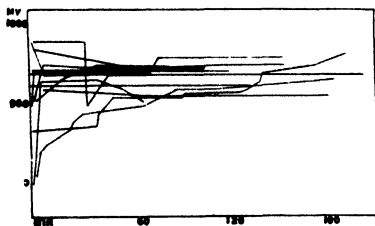


Fig 2

Time-potential curves obtained on different days with an 83μ capillary, No. 5

inch glass plates supported on sulphur blocks. In the latter part of this work, when we came to a comparison of the smallest capillaries with larger, a large and a small were mounted on the same holder and the determinations on the two were carried out simultaneously. The technical difficulties of the preliminary treatment and of the potential measurements increase enormously as the capillary diameter falls below 15μ ; with this arrangement we had a

control determination with a dependable capillary for each determination on a very small capillary. In this way adventitious departures from standard conditions could be recognized. With every determination on the small capillaries, nos. 24, 26, 28, 36, 38, 40, 42 and 46 a simultaneous determination with a 110μ capillary which gave essentially reproducible potentials was carried out.

Fig. 2 shows the curves of stream potential against time given by an 83μ capillary, no. 5. Table I gives the data on all the capillaries. Each figure represents the stable or equilibrium value of potential exhibited through most of the course of a 2 to 5 hour run. It is not necessarily either the highest figure obtained or the reading at the end of the experiment, although in many cases the highest and lowest figures in a 5 hour run did not differ by more than 4 or 5 per cent. An inspection of Fig. 2 will make obvious the meaning of the term "stable value."

While it is true that reproducible values are obtained with the larger capillaries and even with those of 10μ diameter, this cannot be said of those with diameters of 6μ or less. It is seen that capillaries nos. 36, 38, 44¹ and 46

¹ This capillary had a circular cross section of 5μ at each end and of 3μ in the middle.

TABLE I
Stream Potentials of Glass Capillaries of Various
Sizes at 60 cm. Hg. Pressure

<i>Capillary No. 3</i>		2-5	945	<i>Capillary No. 14</i>	
Diameter	47.4 μ	2-6	937	Diameter 38 μ	
Length	5.9 cms.	2-10	910	Length 4.61 cms.	
Date	Reading mv.	2-20	944	Date	Reading mv.
		2-21	906	2-18	1010
10-24	938			2-19	983
10-25	900	<i>Capillary No. 7</i>			
10-27	986	Diameter { 67.5 μ 77.0 μ		<i>Capillary No. 15</i>	
10-28	985	Length 3.8 cms.		Diameter { 39 μ 38.5 μ	
<i>Capillary No. 4</i>		Date	Reading mv.	Length 4.31 cms.	
Diameter	83.5 μ			Date	Reading mv.
Length	2.84 cms.	1-16	892	2-14	928
Date	Reading mv.	1-22	903	2-18	920
1-26	1001	1-23	906	2-20	955
1-29	980	1-26	935	2-23	890
1-29	930	1-27	960	2-25	948
1-30	930	1-28	920	2-26	930
2-2	900	1-28	902		
2-4	930	1-29	914	<i>Capillary No. 16</i>	
2-4	890	1-30	911	Diameter { 112 \times 125 μ 110 \times 123 μ	
2-5	914	2-2	903	Length 5.03 cms.	
2-5	900	2-3	945	Date	Reading mv.
2-6	898	2-4	975	2-6	1012
		2-5	915	2-9	1062
		2-6	910	2-10	1051
		2-9	974	2-11	1000
		2-11	950	2-12	1025
<i>Capillary No. 5</i>				2-13	1030
Diameter	83 μ	<i>Capillary No. 12</i>			
Length	4.6 cms.	Diameter { 85 μ 84 μ		<i>Capillary No. 17</i>	
Date	Reading mv.	Length 5.88 cms.		Diameter { 105 \times 120 μ 105 \times 120 μ	
1-12	964	Date	Reading mv.	Length 8.87 cms.	
1-15	961			Date	Reading mv.
1-16	933	2-13	1000	2-16	1071
1-17	924	2-14	1026	2-18	965
1-17	905	2-16	1060		
1-23	943	2-19	1002		
1-24	948	2-21	1015		
2-3	940				
2-4	947				

TABLE I (Continued)
Stream Potentials of Glass Capillaries of Various
Sizes at 60 cm. Hg. pressure

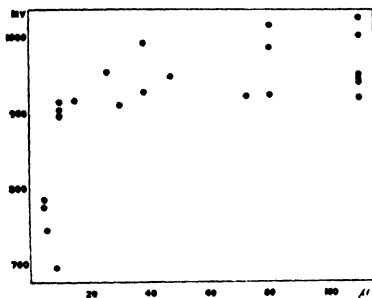
2-19	995	<i>Capillary No. 20</i>		3-11	915
2-21	1020	Diameter	37.8 μ	3-13	930
2-23	1000		38.4 μ	3-14	905
2-26	990	Length 4.4 cms.		<i>Capillary No. 23</i>	
<i>Capillary No. 18</i>		Date	Reading mv.	Diameter 110 \times 118 μ	
Diameter	84.2 \times 84 μ	2-25	975	Length 7.0 cms.	
	84 \times 84 μ	2-27	980	Date	Reading mv.
Length 4.23 cms.		3-2	920	3-5	922
Date	Reading mv.	3-5	967	3-6	938
		3-12	917	3-7	970
2-24	1035	<i>Capillary No. 21</i>		3-10	920
2-26	970	Diameter	105 \times 112 μ	3-11	920
2-28	970		108 \times 115 μ	3-12	1020
<i>Capillary No. 19</i>		Length 5.0 cms.		3-13	1065
Diameter	118 \times 110 μ	Date	Reading mv.	3-16	920
	118 \times 110 μ	3-4	1022	3-17	927
Length 6.3 cms.		3-6	945	3-18	910
Date	Reading mv.	3-9	950	3-25	950
2-24	1048	3-13	1029	3-26	990
2-25	1000	3-14	1049	3-30	926
2-26	947	3-30	900	4-2	898
3-5	922	4-1	932	4-7	970
3-6	987	4-7	911	<i>Capillary No. 24</i>	
3-7	1010	4-8	900	Diameter	15 \times 16 μ
3-9	957	4-14	910		14.6 \times 15 μ
3-11	970	4-17	1020	Length 1.49 cms.	
3-12	975	5-6	900	Date	Reading mv.
3-14	950	5-7	899	3-6	920
3-27	895	5-9	900	<i>Capillary No. 25</i>	
4-3	920	5-25	920	Diameter	108 \times 115 μ
4-7	920	<i>Capillary No. 22</i>			Length 6.15 cms.
4-9	940	Diameter	25 \times 27 μ	Date	Reading mv.
4-10	940		30 \times 32 μ	4-16	920
4-11	960	Length 2.03 cms.		4-24	920
4-13	955	Date	Reading mv.	5-5	940
4-22	915	3-4	948	5-6	910
4-23	895	3-6	890	5-9	919
		3-9	900		

TABLE I (Continued)
Stream Potentials of Glass Capillaries of Various
Sizes at 60 cm. Hg. Pressure

<i>Capillary No. 26</i>		<i>Capillary No. 36</i>		<i>Capillary No. 42</i>	
Diameter 10μ		Diameter 8.8μ		Diameter $\begin{cases} 9.5 \times 10.7\mu \\ 9.5 \times 10.5\mu \end{cases}$	
Length .7 cms.		Length 1.12 cms.		Length .74 cms.	
Date	Reading mv.	Date	Reading mv.	Date	Reading mv.
3-10	860	3-30	700	4-9	900
<i>Capillary No. 28</i>		<i>Capillary No. 38</i>		4-10	910
Diameter $\begin{cases} 10.5\mu \\ 10\mu \end{cases}$		Diameter $\begin{cases} 6\mu \\ 5.6\mu \end{cases}$		4-11	900
Length .8 cms.		Length 1.1 cms.		4-20	915
Date	Reading mv.	Date	Reading mv.	4-21	908
3-17	900	4-2	750	<i>Capillary No. 44</i>	
3-18	900			Diameter $\begin{cases} 5\mu \\ 3\mu \text{ in middle} \end{cases}$	
3-19	930			Length .7 cms.	
3-25	930			Date	Reading mv.
3-26	933			4-9	790
<i>Capillary No. 30</i>		<i>Capillary No. 40</i>		<i>Capillary No. 46</i>	
Diameter $26 \times 26.5\mu$		Diameter $\begin{cases} 9.6 \times 9.6\mu \\ 10.0\mu \end{cases}$		Diameter $\begin{cases} 4\mu \\ 4.5\mu \end{cases}$	
Length 1.5 cms.		Length 0.8 cms.		Date	Reading mv.
Date	Reading mv.	Date	Reading mv.	4-14	730
4-1	950	4-3	900	4-28	850
4-7	960			5-4	760
4-8	960				

with diameters of 8, 5.8, 5 and 4.5μ respectively, show somewhat lower stream potentials than do the larger capillaries. Several possible sources of error enter into the determinations on the small capillaries, all of which tend to make the readings too low. First, due to the very high resistances, amounting to many thousand megohms, the danger of short circuiting is greatly increased. We have taken every precaution to prevent this and are certain that the leakages are not more than a few per cent of the potentials measured. Second, the difficulties of an adequate and constant preliminary treatment are much greater with the smallest capillaries. One cannot have the same degree of confidence that a thorough steaming process has been carried out that one has with larger capillaries. Third, there is greater danger of a low potential due to partial mechanical obstruction. Obstructions cannot be detected by determinations of the rate of flow through these capillaries; one must depend upon the behavior of the galvanometer deflections for information as to the potency

of the capillary. The appearance of an obstruction could of course be detected by measurements of the capillary resistance but we are not yet ready to report on resistance determinations in the smallest capillaries. Since we have obtained with these extremely small capillaries potentials only 10 to 15 per cent lower and with the 10μ capillaries potentials quite as high as with the large, we feel that as technical perfection is more nearly approached it will be possible to get the same potentials from the smallest capillaries as from the large. This is only an opinion; further work may show that under no circumstances will the potentials of the smallest capillaries be quite as high.



should be lower still, only a few per cent of that observed with large capillaries. The fact that the stream potential is not so lowered means that the previously drawn conclusions as to surface conductance at glass-solution interfaces are incorrect, that there was some fundamental difference in the condition of the glass surfaces in McBain's experiments and in ours, that the classical equation for stream potential does not hold in such small capillaries, or that some other factor or factors, as ζ -potential or dielectric constant, is changed in these capillaries in such a way as to compensate for the increased conductivity and maintain an unchanged stream potential.

The first step in the attempt to answer these questions is the direct measurement of the conductance in these capillaries. This work has been started but is yet in the early stages. We have on several occasions observed that a capillary of 10μ or smaller showed a normal stream potential and that in the course of a few days this fell off sometimes down to zero. On other occasions we have seen the potential of a very small capillary rise in the course of a few days from only a few millivolts to the normal level. The question arises whether these changes are due to changes in surface conductance. It is our purpose to follow the capillary resistance through such cycles. In the only cases where we have determined the resistance of very small capillaries showing little or no stream potential the resistance has been infinite, i.e., many times the calculated. This has confirmed our suspicion that these capillaries were mechanically obstructed.

We have been able to measure the resistance of some of our larger capillaries. One would not expect a surface conductance in an 80μ capillary to be so important as in a 5μ . We have employed two modifications of the same method for the resistance measurements. A known high resistance is put in series with the capillary and the P.D. across the resistance is measured by the same technique employed in the stream potential determinations. To measure the capillary resistance while the pressure is on the stream potential itself is utilized as the source of E.M.F. To measure the resistance of the resting capillary a dry cell of known voltage is put in series with the capillary and known high resistance. Knowing the standard high resistance, the E.M.F. and the P.D. across the known resistance, the resistance of the capillary can be calculated. For capillaries with resistances up to about 1500 megohms a 47 megohm "Electrad" resistance was used as the standard. Its resistance was determined by two methods, on a Wheatstone bridge using one dry cell and a high sensitivity galvanometer, and by the leakage of a charged condenser through the resistance. The condenser was a 0.10 mf. mica; it was charged by applying 300 mv. from the potentiometer circuit. It will be noted that neither method uses a high voltage. The two methods agreed to within 3 per cent; the value of this particular Electrad resistance has been determined many times and found to fluctuate a few per cent around 47 megohms; it is therefore redetermined on each day of capillary resistance determinations. The same method may be applied to the smaller capillaries but requires

standard high resistances of from 1,000 to 15,000 megohms, depending on the size of the capillary. Preparation of these resistances has delayed our work with small capillaries.

We have a few data on resistances of four larger capillaries, 74, 80, 80 and 84 μ in diameter. The observed resistance is always less than the calculated. We must state here that possibility of error from these determinations is considerable. Our standard high resistance, 47 megohms, was so low that the P.D. across it was only a small fraction of the total E.M.F. The calomel electrodes did not remain completely isoelectric over a long period; fluctuations of a few millivolts were insignificant in measuring stream potentials of 900 mv. or more but would be very important in measuring an 18 or 20 mv. drop across the standard high resistance. Unfortunately we neglected to determine our electrode potentials often enough during these measurements. These difficulties will not obtain in future work; standard resistances more nearly those of the capillaries are being prepared. These preliminary measurements are introduced here to show that variations in stream potential are not correlated with variations in resistance. For these measurements no attempt was made to obtain reproducible potentials; it was rather our purpose to permit changes in the state of the capillary which would effect changes in stream potentials and to see if these were due to changes in conductance. As is seen in Table II, there is no correlation between stream potential and conductance.

TABLE II
Resistance Measurements

Diameter μ	Length cms.	Stream Potential mv.	Observed Resistance ohms $\times 10^6$ /cm.	Calculated Resistance ohms $\times 10^6$ /cm.
80	3 0	726	2.14	3.12
		670	2.07	
		700	2.02	
		835	2.17	
74	5.9	960	2.54	3.6
		870	2.35	
		900	2.39	
		940	2.69	
		887	2.53	
		984	2.43	
84	5.94	900	2.43	2.81
		781	2.35	
		785	2.34	
		720	2.36	
		650	2.42	
		592	2.58	
		572	2.40	
		732	2.45	
	2 84	746	2.38	
		390	2.38	
		700	2.6	
		700	2.6	
80	3.11	700	2.6	3.12

Thus, the 84μ capillary on one occasion had a potential of 900 mv. and a resistance of 2.43×10^8 ohms/cm. and on another occasion a potential of 390 mv. and a resistance of 2.38×10^8 ohms/cm. The drop in stream potential was not due to an increase in conductance; apparently the effective ζ -potential has decreased.

According to the data in Table II we find a significant surface conductance in capillaries as large as 80μ , even when no allowance is made for the "pinch effect." These data, as stated above, may very well be in error so far as absolute values are concerned. If there is a significant surface conductance it should be more significant in 10μ capillaries. If this is true we have the apparent anomaly of a normal stream potential and an increased conductance. If further work reveals that there is a high surface conductance in these small capillaries with normal stream potentials it will mean either that the stream potential equation does not hold or that compensatory changes in some other factor or factors permit a constant stream potential.

The bearing of these results on the membrane hypothesis is still not clear. No great difference in potential with change in size has been brought out but even our smallest capillaries are, of course, far larger than the largest pores in a cellophane membrane. Our experiments have not answered definitely whether pore size is a factor, but experience with untreated glass capillaries leads to the conclusion that inequality of stream potentials across the various pores should be the rule rather than the exception. The conception of short circuits through relatively inactive pores to explain the absence of a stream potential across a cellophane membrane still appears reasonable but has not been experimentally established. We would call attention to two papers by Söllner,¹ which have come to our notice since our first communication was read last June, in which he uses essentially the same conception to explain abnormal osmosis.

Summary

The stream potentials exhibited by pyrex capillaries of diameters ranging from 4.5 to 110μ through which 0.0005 M KCl is forced at 60 cm. of Hg. pressure have been measured. A standardized steaming treatment has been established which permits satisfactorily reproducible results. Stream potential is independent of capillary diameter down to 10μ . Capillaries with diameters between 4.5 and 8μ have shown somewhat lower potentials; whether this is a real phenomenon or due to technical difficulties is not yet clear. On the basis of reports by other workers on surface conductance at glass-solution interfaces one should expect a much lower stream potential with our smallest capillaries. The explanation of the absence of the predicted falling off in stream potential with the smallest capillaries will depend upon the results of resistance measurements now under way.

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¹ Z. Elektrochemie, 36, 36, 234 (1930).

MINERAL FLOTATION

BY ARTHUR F. TAGGART

Introduction

Appearing as it did in an art (ore dressing) that utilized mechanical principles almost exclusively in its operation, and developing empirically, flotation was first investigated from the purely physical standpoint. And because the controlling phenomena are almost wholly chemical, progress in understanding was correspondingly slow. Since the chemical aspect of the process has been recognized, however, investigation has made rapid strides, and a sufficient background of knowledge has

now been built up so that methods of research and control founded on established chemical principles may be confidently applied.

There are a number of so-called flotation processes, but the essential differences between these methods narrow down on analysis, leaving two fundamental groups, which may be designated as bubble-column processes and pulp-body processes respectively. The former are the simpler both in respect

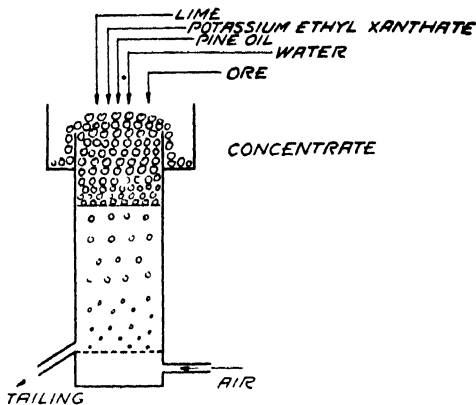


FIG. 1

Diagrammatic sketch of bubble-column flotation

to operation and investigation, and may be taken as the type for study.

Fig. 1 presents diagrammatically a common bubble-column operation of the present day. Finely ground (minus — 0.2-mm.) sulphide ore mixed with three to four times its weight of water (the mixture is called *pulp*), is flowed continuously into one end of a porous-bottomed trough-like tank. Lime (1 to 4 lb. per ton of ore) has usually been added to the pulp during grinding. Xanthate (0.05 to 0.2 lb. per ton) goes in from a few seconds to a few minutes before the pulp reaches the flotation cell. Pine oil (0.05-0.1 lb. per ton) is introduced at the head of the cell with the stream of pulp. Air is blown in at the bottom, as pictured. A watery froth carrying the bulk of the sulphide mineral (*concentrate*) overflows continuously; the residual impoverished solid with water (*tailing*) also discharges continuously. Buoyancy of the concentrate particles is brought about by forming aggregates thereof with the air bubbles. The lime and xanthate are added to induce this aggregation. Pine oil imparts stability to the bubbles.

Levitation

In froth flotation of sulphide ores the material that floats is the specifically heavier part. This fact excludes the hypothesis that the overflowing solid in the bubble-column cell is lifted by the rising fluid current induced by the air bubbles. For if such were the mechanism, it would be the specifically lighter material that overflowed.

The particles that overflow are lifted by the bubbles. The reason for the bouyancy of the bubble-solid aggregates is, of course, simple displacement. But the means of attachment of bubble to particle is more difficult to determine. There will occur to the mind of anyone whose recollection runs back to his days in physical laboratory the floating-needle experiment, and—not too

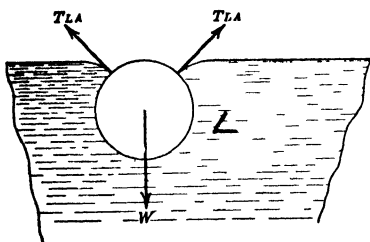


FIG. 2
A floating needle

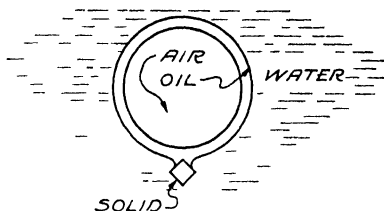


FIG. 3
Probable arrangement of phases in a bubble column

literally—the same force that held the needle at the surface of the water there holds the concentrate particles in the bubble films. But it is distinctly questionable whether tension of the same interface is effective in both cases. With the needle it was the air-water surface tension, the needle being in equilibrium under the forces pictured in Fig. 2. It is doubtful, however, whether the concentrate particles in a bubble-column froth are in the air-liquid interface; the weight of the evidence is that they are in the interface between a layer of oily liquid forming an inner sheath of the bubble, and the surrounding water. (See Fig. 3.)

Experimental

1. A portion of bubble film may be picked up by dipping a wire ring $1/4$ to $3/8$ -in. diameter into the bubble. If fresh film carrying solid is thus obtained and examined immediately at 30- to 50- \times magnification, the air-liquid interface appears unbroken. After 30 seconds to a minute liquid appears to draw away from points of the solid particles that project toward the lens, and these points then protrude into the air. The conclusion would seem to be that the method of examination is capable of distinguishing between an unbroken liquid-gas contact and a solid-gas contact, and that it is the former that exists at the surface, and probably, therefore, in the body of an operating bubble column.

2. A bubble column will not operate if an oil or other substance capable of forming an oily film on the bubbles is missing.

3. The force of the adhesion between solid particles and bubbles in a bubble-column is apparently less than that between the solid particles and the bubbles in a pulp-body operation. (See p. 143.) In this latter type of process the solid particles are in the air-liquid interface. (See Experiment 30, p. 146.) Since the air-liquid surface tension of the

overflow liquid in a properly conducted frothing operation is normally upwards of 60 dynes per cm., while the interfacial tension of the oil used against water is probably not over half that figure, the observed difference in adhesion is significant.

4. If, say, 1 cc. of a non-frothing oil such as Nujol is placed with 5 gm. of a deslimed sulphide ore and 25 cc. of water in a 50-cc. test-tube and the latter closed with a clean cork (not with the thumb) and shaken vigorously three or four times, the oil is broken up into relatively coarse droplets, and microscopic examination (10- to 20-x) shows that sulphide particles are held suspended by these droplets, as is the particle in Fig. 3. If now a minute amount of a spreading oil (see below) is introduced and the tube is again shaken three or four times, the oil droplets differ from those before observed in that, in addition to their solid load, many or all contain air bubbles. These now are obviously in the exact condition pictured in Fig. 3, except that the oil sheaths are relatively thicker. Successive shakings produce progressive thinning of the oil sheaths and more bubbles. Final prolonged shaking will, if conditions are right, produce a mineral-carrying froth of such great bubble surface that the oil layers are so thin as to be invisible under the magnification prescribed. But since the solid particles were seen to be carried, so long as the oil sheath was visible, at the oil-water interface, and since we know, from surface-tension considerations, that the oil film persists, the conclusion seems reasonable that the adhering solid particles remain in the oil-water interface.

Frothing

If pine oil is eliminated from the operation pictured in Fig. 1, all other conditions remaining the same, the volume of the column of bubbles overlying the body of pulp will be materially smaller, so much so that there will be no overflow, and visual evidence of concentration (see p. 143) will be lacking. The pine oil has two functions; it stabilizes the bubble films so that they persist and build up to the overflow level, and it furnishes the oil sheath around the bubbles for holding the solid particles.

The frothing action of a reagent involves spreading of the reagent at the air-water interface, with consequent increase in concentration of the reagent in overflow as compared with the residual liquid. Only those substances which spread are useful frothers. They spread because they lower the surface tension of the air-liquid interface. Their effectiveness as frothers is in proportion to the intensity of their effect on this surface tension, when present in low concentrations. Soluble substances that raise the surface tension of water also have been reported to exert some frothing effect, but are not practical frothers in the flotation art.

Experimental

5. Fig. 4 presents results of surface-tension measurements on overflow and residue liquids from a series of flotation operations in a pneumatic cell, using pine oil as a frother and no other reagents added. Fig. 5 shows the relation between weight of overflow (solid plus water) and difference in surface tension between overflow and residue. The range in quantities of pine oil present, between 20 and 40 mg. per l., is the usual operating range for this reagent, hence it is to be concluded that a reagent that will lower the surface tension between 3 and 5 dynes per cm. at such low concentrations has requisite frothing power.

6. Solutions of sulphuric acid, acid sodium sulphate, and of sodium chloride and sulphuric acid, of 1 to 2 per cent strength, have been used as sole reagents in froth flotation of certain high-grade sulphide ores. The effect of these inorganic substances on the surface tension of the solutions at the concentrations used is an elevation of the order of a dyne per cm. It is distinctly doubtful whether the froths obtained were due to the inorganic

reagents directly. Sensible amounts (from the flotation standpoint) of lubricating oils and greases are present in practically all ores that have been machine mined and milled to flotation size. Such relatively strong inorganic solutions as those under discussion, hot, as they were used, would tend to dislodge such oils from the gangue of constituents of the ores, break up the soaps in the greases, and thus free organic reagents for frothing and collecting, and it is, in all probability, these that were effective.

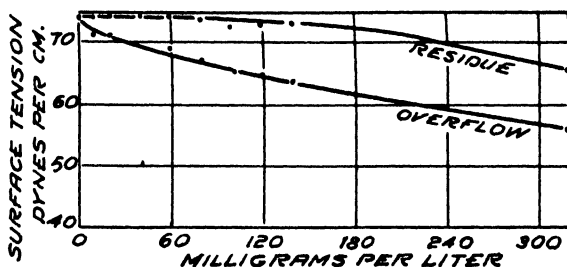


FIG. 4

Surface tensions of overflow and residual liquors from a bubble-column operation

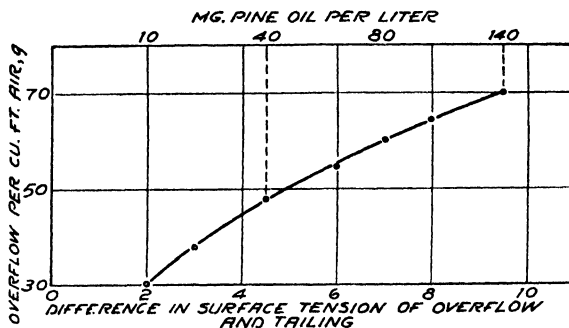


FIG. 5

Frothing on change in surface tension

The way in which the frother functions is best understood by analysis of the ideal experiment pictured in Fig. 6. ABCD is a U-shaped loop of wire set with legs vertical, and EF a straight wire of weight W making frictionless contact therewith. The shaded portion is a liquid film. At equilibrium $W = 2(BC)T$, where T is the air-liquid surface tension per unit length of film. If $W > 2(BC)T$, and the film is a pure liquid, EF will move downward until the film breaks; if $W < 2(BC)T$, EF will be pulled upward until it makes contact with BC. If, however, the liquid is contaminated with a surface-tension-lowering reagent whose concentration in the surface of the film at a given instant is considered to be represented by the spacing of the shading lines, and if, at the same instant W exceeds $2(BC)T$, EF will move downward as before. In so moving it will increase the surface and thereby increase momentarily, at least, the spacing of shading lines, which is to say, the molecules of the contaminant. As a result, since the extent of lowering of the surface tension is

roughly proportional to the concentration of contaminant in the surface, surface tension will increase and tend to counterbalance the excess load. And *vice versa*. Thus a contaminated film has the capacity for automatic adjustment to external strains within its ultimate strength, and in this way its life is lengthened, which is to say that the contaminant adds stability to a froth made with the liquid.

Fig. 7 shows typical surface-tension curves of a good frothing agent (A), a poor frothing agent (B), and an inorganic salt which has some frothing power (C), all with water against air. At low concentrations a change from con-

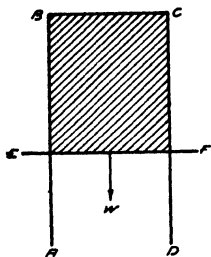


FIG. 6

centration D to concentration E in the surface of the film produces a large change in surface tension with A, while the changes with B and C are almost negligible.

Excluding inorganic compounds, frothing agents have reasonably definite chemical characteristics. The best fall, in general, in the class of organic compounds rated as slightly soluble, and their chemical structure is characterized by a hydrocarbon aggregate of relatively high molecular weight carrying an oxygen-bearing polar group. An investigation¹ of the frothing power of some 500 organic compounds of all classes showed an average of 5.5 carbon atoms in the hydrocarbon aggregate of the molecule of good frothers; the principal polar groups, in order of decreasing frequency of occurrence, were OH, CO, COOH, CONH, COO, and COC; and the solubility centered around 1000 mg. per l.

The molecule of a frother may be pictured as composed of two different and distinct parts, so far as its reactions toward water and effect on air-water surface tension are concerned. The hydrocarbon part of the molecule resists incorporation with water; the oxygen-bearing part is water-avid and tends to surround itself with water molecules. If the oxygen-bearing part predominates, the entire molecule is drawn into the water and the solubility is high; if the hydrocarbon part predominates too greatly, solubility is very low. With a suitable balance, however, the molecules accommodate themselves readily to the air-water interface, so oriented that the water-avid oxygen-bearing group is in the water and the hydrocarbon in the surface. In such a position the large hydrocarbon groups may be considered as crowding the water molecules apart, thus lessening their mutual attraction, which is to say the surface tension of the liquid, while at the same time no compensating molecular attraction between adjacent molecules of water and hydrocarbon is set up. This is inferable from the low solubility of hydrocarbons in water. The mutual attraction between the organic molecules in the surface (surface tension) is less than that between water molecules. Furthermore these organic molecules are separated by water molecules in the same way as water molecules are crowded apart by organic, so that the presence of the organic molecules in the surface does nothing to compensate for the decrease in surface tension of the water that these molecules cause.

¹ Taggart, Taylor and Ince: Tech. Publ. 204, Am. Inst. Min. Met. Engrs. (1929).

The best frothers so far discovered are alcohols and ketones of the hydro-aromatic series, e.g., terpineol, menthol, eucalyptol, borneol, camphor and the like, and more particularly essential oils containing high percentages of such substances. The straight hydrocarbon constituents in these natural oils are caused to spread at bubble surfaces by reason of the frothing agent dissolved in them, and these oil films seem to furnish better collecting interfaces than films of soluble agents alone. Crude cresylic acid is similarly a more effective frother than highly purified cresols.

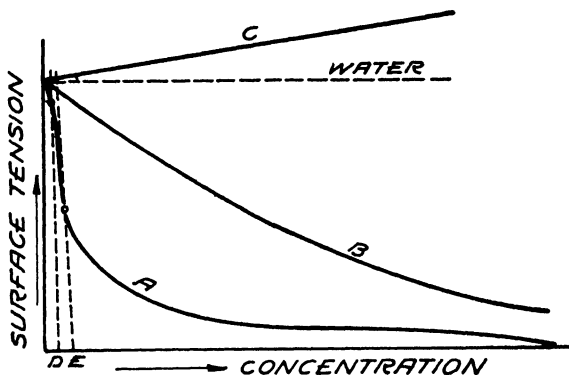


FIG. 7
Surface-tension curves

The frothing effectiveness of the individuals in the lower ends of homologous series increases with increase in molecular weight, which is to say with decrease in solubility. The probability is that frothing-concentration curves for all such series pass through maxima at moderately low solubilities. In all series effective frothing power is substantially zero for the members that are miscible with water in all proportions, and likewise for those members that are actually substantially insoluble. Ordinary handbook ratings of insolubility are not, however, to be accepted as final in this connection, since many reagents used in flotation and dependent for their effectiveness on their solubility are rated in the hand books as insoluble. The proportions in respect to the water in which the reagents are added in modern flotation ranges from 1 part in 10 000 to 1 part in 100 000 commonly, and only a portion of even these minute quantities seems to be necessary to be in solution at any one time in order to function effectively. Hence no higher member of a promising series is to be rejected without trial on handbook evidence alone.

Experimental

7. Table I shows results selected from a series of carefully comparative frothing tests with the lower members of two allied homologous series. Cresol, with the additional methyl group and resultant lower solubility, is more active than phenol. Xylidin is superior to toluidine, which is superior to aniline. Aniline is less active than its analog, phenol, and toluidine than cresol, indicating the amino group less water-avid, and therefore of less powerful and rapid orienting effect than hydroxyl. Benzene itself has no frothing effect.

TABLE I
Results of Comparative Frothing Tests

Reagent	Conc'n. mg. per l.	Total overflow, gm. per cu. ft. of air	Rated solubilities, mg. per l.
Phenol	200	33.7	60 000
Cresol	25	38 (a)	27 000 (b)
"	200	64.9	"
Aniline	200	27.3	31 000
Toluidine	25	23.0	sl [o]
"	200	38.0	7400 [p]
Xylidin	25	28.6	v. sl.

(a) By interpolation. (b) Mean of [o] and [p].

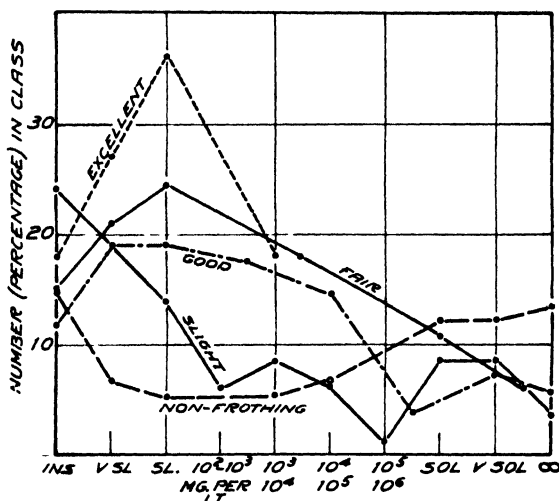


Fig. 8

Owing to difficulties in making quantitative measurements of froth-producing ability, and consequent lack of orderly investigation of various chemical series of frothers, no definite location of the solubility range corresponding to maximum frothing ability can be set forth. Fig. 8 is an approach to quantitative estimation, however, presenting graphically the results of assessments by experienced operators of comparative frothing powers of some 250 chemical compounds of the frothing type.¹ Eleven of these rated excellent, 68 were good, 114 fair, 81 showed slight frothing power, and 74, which had polar-nonpolar structure with oxygenated polar groups, failed. The groups rated excellent, good and fair all show the bulk of the compounds to lie in the solubility range from very slight to slight; here lie also a considerable percentage of the slight frothers. The bulk of the failures lie, as is to be expected, in the insoluble and highly soluble ranges.

The solid-loaded films of a froth-flotation operation are enormously more persistent than any liquid films produced with the same reagents present, but no solid. The reason for the increase in strength is increase in film viscosity

brought about by the solid. The effective phenomenon is probably the same as that in childhood's mudpies. Some heavily-laden froths are so stiff that the froth structure survives dehydration. The closely crowded solid particles key each other into position, while the very thin inter-particle liquid films have characteristically high viscosities.

Experimental

8. If a bubble column made with water and a small amount of frothing agent is stabilized, by regulating the air supply, at a given height in a pneumatic flotation apparatus, addition of a small quantity of finely pulverized solid will cause the height of the column to increase markedly.

9. Float a steel needle on a beaker of clean water (beaker diameter 2 to 3 times the needle length) and place a chip of paraffin wax near the beaker wall. The needle may be rotated about a vertical axis by means of a magnet without moving the chip. Next dust the surface with fine dry solid. Galena that has been washed in a dilute benzol solution of oleic acid and air-dried is good for this purpose. With the surface thus coated with solid the paraffin chip will move when the needle is rotated.

Collection

Potassium ethyl xanthate in the operation pictured in Fig. 1 is known as a collector. If it is omitted and no substitute added, the amount of sulphide mineral overflowed is markedly decreased, and the assay value of the concentrate likewise diminishes greatly. Numerous substitutes for potassium ethyl xanthate are known, ranging from homologous xanthates through chemically similar organic compounds to straight hydrocarbon oils. The oils come first chronologically, but we are largely ignorant concerning the reasons for their action; the xanthates and their like are the modern collectors, and considerable is known concerning the mechanism of their behavior.

Oils

Experiment shows that insoluble highly refined hydrocarbon oils wet sulphide minerals preferentially in the presence of water, while the reverse is the case with gangue minerals. Excluding the possibility of chemical reaction between oil and sulphide—and such exclusion is almost forced, if the oil is of the class described—the action seems to be explicable only on grounds of the interfacial tensions involved. On this basis, applying the law of least energy, it would appear that the energy of an oil-sulphide interface is less than that of a water-sulphide interface, while as respects quartz the relative magnitudes of the interfacial tensions are reversed. We cannot, of course, confirm this reasoning by direct measurement of tensions, and the suggested explanation remains, therefore, a matter of inference.

Experimental

10. If a sulphide-bearing ore is ground in the presence of water with a highly-refined paraffin-hydrocarbon oil (white paraffin, Albolene, Nujol, liquid white vaseline, or the like), and the sulphide mineral is subsequently separated from the gangue and both are analyzed for oil, it is found that the percentage of oil in the sulphide fraction is much greater than in the gangue fraction.

11. If drops of Nujol are brought into contact with plane-surfaced particles of quartz and of galena immersed in water, the droplets spread out into adherent sub-hemispherical segments on galena while on quartz they assume substantially spherical shape and are practically non-adherent. A variety of oils may be substituted for the refined hydrocarbon oil without changing the experimental observations, e.g., creosotes from various tars, essential oils, and liquid fatty oils and fatty acids of relatively high molecular weight. But many, if not all, such substances contain ingredients that might conceivably react chemically with the surface constituents of the sulphide particles; consequently, with respect to such oils, the possibility of chemical action may not be excluded. They probably act, to some extent at least, like the chemical collectors.

Chemical Collectors

The class of reagents of which the xanthates are typical is known in flotation terminology as chemical collectors. Study of a large number of these substances, empirically discovered, discloses that the best fall into a group characterized by the presence in the molecule of a multivalent amphoteric element, combined in negative low-valent or reduced state. Examples are potassium ethyl xanthate in which the S in the SK group is the characteristic element; thiocarbanilid, in which the S, probably in the tautomeric HS— form is the essential atom; and diazo-amino-benzene, containing the active group $-N=N-NH-$.

Experimental

12. In a list of 122 chemical compounds that showed distinct collecting properties on test,¹ there were 193 polar groups. Of these, 98 contained trivalent nitrogen with no other multivalent negative element in reduced state, 26 contained sulphur as the only reduced element, and 13 contained both trivalent nitrogen and divalent sulphur. There were 58 oxygenated groups. The list of polar groups in order of frequency was: NH_2 , 29; N , 22; OH , 19; NH , 18; S , 16; $N=N$, 14; NO_2 , 13; $CSNH$, 12; COC , 8; $COOH$, 7; COO , 6; SH , 5; CON , 4; $N=N-NH$, 4; SM , 4; CO , 3; $-N=NH$, 2; SR , NO_2 , $C \equiv N$, I , NO , NCS , $CSeNH$, each 1. Somewhat more generally arranged, there were 71 amino groups, 17 amides and thioamides, 16 azo and diazo, 16 sulphide, and 11 sulphydrate. The order of frequency is not significant as regards present-day practice. The nitrogen compounds weigh unduly on account of the fact that a large part of the experimentation analyzed was done with a legal slant in which attention was focussed on these substances. Sulphur compounds of the sulphydrate type predominate in practice.

Beside these so-called active groups the chemical-collector molecules must contain a reasonably bulky hydrocarbon portion. The statistical study already cited showed an average of 7 to 8 carbon atoms in this part of the molecule.

The chemical collectors function by forming a substantially insoluble oriented water-repellent film on the surfaces of the minerals to be floated, while at the same time the other minerals remain unaffected. The sulphydrate collectors, which are the only ones that have been investigated with any pretence to thoroughness, form this film by reaction with the surface of the mineral particles that they coat. The reaction appears to be one of ordinary double decomposition, and proceeds to substantial completion because the filming compound formed is more insoluble than either the compound forming the mineral surface in the mill pulp or the reagent itself.

Experimental

13. If a solution of a chemical collector of known strength is shaken with sulphide mineral finely ground in air or in water exposed to the atmosphere, and the solution is then filtered off and tested as to concentration of collector, it is found that collector has been abstracted. This test has been made with α -naphthylamine, potassium ethyl xanthate, diphenyl thiourea, monophenyl thiourea, and thiocresol [p]. Abstraction occurs with galena, chalcopyrite and pyrite but not with quartz or calcite.

14. With potassium ethyl xanthate as the collector and galena as the sulphide, xanthate ion is abstracted from solution and sulphate, sulphite (S_nO_m) and carbonate ions, in total amount stoichiometrically equivalent thereto, are thrown into solution. Potassium remains in solution throughout and lead does not appear in solution. If the galena is ground dry or wet in an atmosphere of nitrogen and the abstraction test is made in the same atmosphere, abstraction is reduced to substantial nullity.²

15. If particles of galena are tested as to their reactions toward air bubbles in the presence of water, before and after soaking in collector solutions, it is found that in the presence of such solutions air displaces water from the particle surfaces and the bubbles adhere, while in the absence of collectors the bubbles do not adhere.

16. While the preceding experiment shows that the collector makes the mineral-particle surface water-repellent, and experiment 14 shows that the surface change is due to metathesis, orientation of the surface coating must be concluded by inference. X-ray crystallography indicates that when oxidation occurs at the surface of sulphide minerals, the original sulphide lattice structure tends to be preserved and that the first change is merely substitution of the oxidized anion for sulphide ion with some corresponding expansion of the lattice at the surface. The metal ions retain their original relative positions. When, therefore, the oxidized anions are replaced by collector anions, it is inferential that the resulting compound is oriented at the sulphide-particle surface with the anion toward the solution.

More evidence lies in the fact that if for diphenyl thiourea, which reacts with oxidized lead compounds at galena surfaces to form the corresponding lead-urea salt, which salt adheres to the galena-particle surface and, inferentially, is oriented with the two phenyl groups toward the water, dihydroxy diphenyl thiourea is substituted, abstraction occurs, but the coated particle is not water-repellent. In other words, the substitution of water-avid polar groups on the water end of the phenyl groups causes the result that inference would predict. The evidence for orientation would, therefore, seem to be as complete as is possible, lacking necessarily the ultimate proof of vision.

17. The following abstraction and collection tests were run with galena dry-ground in air; Diphenyl thiourea, 80% abstraction; water-repellency 100 on an arbitrary scale. Monophenyl thiourea, 83% abstraction, water-repellency 59. Monophenyl urea, 7% abstraction, water-repellency 0. Thiourea, 42% abstraction, water-repellency 0. Urea, abstraction and water-repellency both zero. The thioureas, capable of tautomeric change to the acidic $-SH$ form, all reacted with the oxidized lead coatings on the sulphide particles to form adherent lead salts. The bulky hydrocarbon groups in the phenylated compounds contributed water-repellency. The urea compounds, not acidic toward the lead salts, did not react and were not abstracted and consequently the particle surfaces were unchanged.

It is worthy of note that seleno benzamid (Exp. 12) is an equivalent for thiobenzamid. Phosphorus is to be expected to function similarly, but no useful $-PH$ compounds have been discovered. It was thought at one time that the so-called phosphocresylic type of reagents, formed by treating alcohols with phosphorus pentasulphide might be of this type. But Christman³ gives to the active substance in these mixtures the general formula $(RO)_2P(S)SH$,

² Taggart, Taylor and Knoll: Tech. Pap. 312, Am. Inst. Min. Met. Engrs. (1930).

³ Tech. Paper 17, American Cyanamid Company.

with the phosphorus pentavalent, while analyses made at Columbia University of the product formed by treating crude cresylic acid with P_2S_5 indicate that the active collector is a mixture of the three thiocresols. Compounds of the general type $R\text{TeH}$, $R\text{AsH}$ and RSbH should almost certainly be collectors, although none have been recorded.

It would seem to follow from the fact that collection involves bringing about a precipitation reaction between the collector and the mineral to be collected, and since the precipitate is a compound of an anion from the collector and a base from the mineral, that in order to test the suitability of a given reagent for a given mineral it would be significant, if not sufficient, to mix dilute solutions (25 to 50 parts per million) of the collector and a soluble salt of the base-metal of the mineral in a test-tube, and observe whether precipitation occurs. A considerable number of such tests have been made at Columbia. The most that can be said as yet is that with some collectors and some bases the test-tube indications are sufficient, but that very slightly soluble and very unstable collectors may give solutions that are so cloudy initially that precipitation with the base-metal salt is not distinguishable, and that certain other collectors require the pH to be within such narrow limits for precipitation—the limits unknown beforehand—that a negative result is not necessarily reliable. On the other hand, the discovery of diphenyl thiocarbazid for differential flotation of siegenite (cobalt-nickel sulphide) from chalcopyrite was determined in the Columbia laboratory by the test-tube method before a single test was run in the flotation cell.

Conditioning

If lime is left out of the operation pictured in Fig. 1—assuming the ore to have been one requiring lime—the amount of metal in the overflow (*recovery*) would have been less and the probability is that the assay of concentrate would have been lower also. Lime in such an operation is known as a conditioning agent. Such reagents perform one or more of a number of known functions, and may also play other parts as yet unknown.

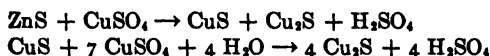
The known functions of conditioning agents are:

1. To react with the mineral to be floated in such a way that the resultant salt forming the modified surface will react with the collector to form an adherent water-repellent film. Such reagents are sometimes called *activators*.
2. To react with one of the minerals in the ore in such a way that while before treatment it would have reacted with the collector and floated, after treatment it will not. Such reagents are sometimes called *depressors*.
3. To react with and remove, by precipitation or otherwise, salts in solution which, if present, would react with other flotation agents. Reagents that act thus may be called *conserving* or *protective agents*.
4. To control the behavior of the finest ("slime") gangue particles toward the minerals to be floated. Since such reagents cause readily visible changes in the degree of dispersion of the slimes they are called *dispersion agents*.

5. To bring and maintain the pH of the solution within such limits that the chemical reactions involved will proceed with maximum velocity and to the requisite extent.

Experimental

18. The best known activator is copper sulphate, which is used to enhance flotation of sphalerite. When used with a collector such as potassium ethyl xanthate, the sequence of known facts is as follows. Powdered sphalerite abstracts copper ion from solution and the concentration of zinc ion in the solution increases correspondingly. According to Zies, Allen and Merwin⁴ the reactions are:



Both reactions are accelerated toward the right by the presence of lime. The concentration of CuSO_4 solution in flotation is normally about 1 part in 8000 to 10 000. With concentrated solutions and high temperatures the sphalerite is visibly altered.

The cuprous-sulphide coating on the sphalerite surfaces oxidizes rapidly and the oxidized surface reacts with xanthate to form an adherent film of insoluble, water-repellent cuprous xanthate. These conclusions are evidenced by abstraction of xanthate ion and corresponding appearance of sulphate ion in solution.

Gaudin and Anderson⁵ report the use of lead salts to activate malachite, and attribute their utility to exchange of the base-metal ions. Gaudin, Glover, Hansen and Orr⁶ report activation of calcite by copper and lead nitrates when soaps are used as collectors.

The early use of sulphuric acid as an activator with insoluble oily collectors probably involved solution, by chemical reaction, of oxidation products from the mineral surfaces, leaving clean sulphide surfaces available for preferential wetting by oil.

19. Depressors react with the minerals that they affect to form adherent salts less soluble than the salts that the collector would form with the base of the mineral. Alkali cyanides are typical depressors for sphalerite. Powdered sphalerite abstracts cyanide ion with corresponding discharge of sulphate and sulphite ions into solution. Zinc cyanide is less soluble than zinc xanthate, hence xanthate ion is not abstracted when the treated sphalerite is immersed in xanthate solution.

Copper sulphate reactivates the depressed sphalerite. Copper sulphate decomposes zinc cyanide, forming first the soluble cuprocyanide ion; further copper sulphate then reacts with the sphalerite as in Exp. 18.

Chromates similarly depress galena, but no reactivating reagent is known.

Organic depressors of the type of urea and the hydroxyphenyl thioureas could be used for metals with which they form adherent salts. The only case of such usage that has come to the writer's attention, however, is that of lactic acid to depress mica in a sulphide flotation operation. It is inferential, without further evidence, that the hydroxy-acid structure of the lactic acid furnishes a reactive polar group that bonds the reaction product to the mica surface, leaving the other water-avid polar group to repel air bubbles.

Certain colloidal materials like glue, albumen, tannin, saponin and the like act as depressors. Their action and control are not, however, understood.

20. Iron salts in solution apparently react with α -naphthylamine and with benzidine, probably also with xanthate, to form compounds of distinctly less effectiveness as collectors than the original reagents. Lime is added to such pulps as a protective agent. It removes the iron by precipitation as hydroxide. Soluble copper and lead consume xanthates, thio-cresol and the like by forming insoluble precipitates. Soluble sulphides clear up such solutions effectively. When sulphides are used, however, the pulp should subsequently be thoroughly aerated before a sulphydrate collector is added, otherwise the surfaces of the

⁴ Econ. Geol., 11, 408 (1916).

⁵ Tech. Paper 9, (1930), University of Utah.

⁶ Tech. Paper 1, (1928), University of Utah.

sulphide-mineral particles are not sufficiently oxidized to react with the collectors. Thus cerussite or malachite soaked in H_2S water and remaining therein show no water repellence when xanthate or thiocresol is added, but if aerated after sulphidizing and then treated with the same reagents, their water repellent quality is high.

21. If a galena particle is immersed in a pulp made of slimed quartz and distilled water (probably a trace of iron is also essentially present), and the mixture is stirred for several minutes, the galena surface after removal of the particle and washing with distilled water will be substantially unchanged so far as microscopic examination can determine. The quartz particles are dispersed and in Brownian movement. If now lime in an amount equivalent to from 1 part in 8000 to 1 in 2000, reckoned on the water, is added to the pulp and the above treatment of galena repeated, the galena surface becomes heavily coated with quartz particles that do not wash off, and the quartz particles in the pulp are flocculated. With certain other slimes of different mineralogical nature, notably ones that contain considerable portions of kaolin, the behavior toward galena and as regards flocculation is exactly reversed.

Slime-coated sulphide surfaces adhere to gas bubbles with difficulty, if at all. Consequently reagents that control slime behavior thereby control flotation.

The mechanism of slime coating is not thoroughly understood. Ince⁷ published results of migration tests on quartzitic and clayey slimes as well as on galena and sphalerite suspensions which indicated that when coating occurred the slime and sulphide particles were oppositely charged, and that when both were like charged there was little, if any, coating. The obvious conclusion is to consider coating as simple coalescence or flocculation of unlike charged particles. But the flocculation of the slimes themselves concurrent with coating is not consistent with such a conclusion. Furthermore, subsequent work on the charges on the particles has failed to confirm Ince's findings, and indeed has thrown doubt generally on migration results as applied to this class of materials.

Experimental

22. pH control. Gaudin, Haynes and Haas⁸ have published results of a comprehensive investigation of the effects of pH on the flotation of sphalerite, both with collectors alone and with collectors plus an activator. Recoveries with the collectors investigated in the absence of an activator passed through maxima in every case, the pH of maximum recovery being set down after the name of the collector in the following list of those tested: thio-cresol [p], 6.2; iso-amyl mercaptan, 6.4; potassium-n-amyl xanthate, 4-5; di-iso-amyl ammonium di-iso-amyl di-thio carbamate, 7-9; mono-(9.5-10.5) and tri-iso-amyl amine, 7.8; phenyl hydrazine, 6.2; di-phenyl hydrazine hydrochloride 2.5-3.5; and iso-amyl phenyl hydrazine, 5. On both sides of the maxima the recoveries fell off rather rapidly to negligible figures.

The effect of copper sulphate was in all cases to widen the zone of highest recoveries. With the sulphhydrate compounds it additionally raised recoveries in the maximum zone, but with the nitrogen compounds there was no distinct betterment in the maxima.

Thomas⁹ recommends close control of alkalinity to the range from 7.5 to 8 for flotation of metallic gold and silver, when lime is used to precipitate iron and copper salts (protective reagent). He states that higher concentrations of lime cause marked decreases in recovery.

While no data may be cited to prove directly that the zones of maximum recovery above recorded correspond to pH bands of maximum reaction velocity

⁷ Tech. Pap. 195, Am. Inst. Min. Met. Engrs. (1929).

⁸ Tech. Pap. 7 (1930), University of Utah.

⁹ U. S. Pat. 1,802,989 (1931).

between the respective collectors and the mineral-particle surfaces, yet such a conclusion and explanation of the facts seem almost unescapably inferential.

Aeration

The operation pictured in Fig. 1 will not be materially affected if nitrogen or oxygen or hydrogen or illuminating gas is substituted for air. Further, froth flotation may be effected with the reagents or their equivalents added in this same operation, if other methods of introducing gas are employed, e.g., if the pulp is boiled, or subjected to a vacuum, or if a gas such as CO_2 is generated in the pulp by chemical reaction of an acid and a carbonate, or if the pulp is beaten by a rapidly revolving stirrer. But the mechanism of mineral-bearing froth formation thus effected is different from that in the operation pictured.

Experimental

23. If sample tubes are inserted at short vertical intervals along the side of a transverse section of the bubble-column machine shown in Fig. 1, and if simultaneous samples are drawn and assayed and the results plotted, they yield a graph like that in Fig. 9. The interpretation of these data is that all of the concentration that is effected takes place in the bubble column.

Visual examination of an operating machine fitted with glass sides shows that the color of the muddy liquid comprising the bubble walls is the same at the bottom of the bubble column as that of the underlying pulp, while, in the case of an ore containing a dark-colored sulphide and a light-colored gangue, the bubble column becomes greatly darker as the top is approached.

24. Fig. 10 is a diagrammatic cross-section of one unit of a typical agitation-froth machine. B is a deep box, square in horizontal section, connected by a hooded slot with the settling box C. Pulp with reagents enters B and after suitable agitation discharges into C where froth rises to the top and overflows, and impoverished pulp discharges at the bottom, to enter an adjoining unit or be sent to waste.

If, with the ingredients indicated in Fig. 1, an operation in the apparatus of Fig. 10 is sampled along section AA, as in Exp. 23, and the samples are similarly assayed and plotted, the resultant graph is as shown in Fig. 11. This curve is made up substantially of two vertical segments *ab* and *cd* with a discontinuity from *b* to *c*. Interpreted it means that full concentration is complete at the bottom of the froth layer, the bubbles emerge from the pulp body with their selection already done, in other words, concentration has been effected at the bubble surfaces in the body of pulp itself. Since the same operation can be carried on in the agitation chamber alone by closing the outlet slot and stopping agitation after a suitable time, at which time a mass of froth rises immediately to the pulp surface, it may be further concluded that concentration is effected and completed at the bubble surfaces in the agitation chamber of Fig. 10.

25. The mechanism of particle behavior during concentration in a bubble-column operation may be observed directly by examining the pulp in the bubble walls of an operating column in a glass-sided cell using a magnifying glass of sufficient strength (about 10- \times) to resolve individual sand particles. (There is a knack to the observation. The eye must

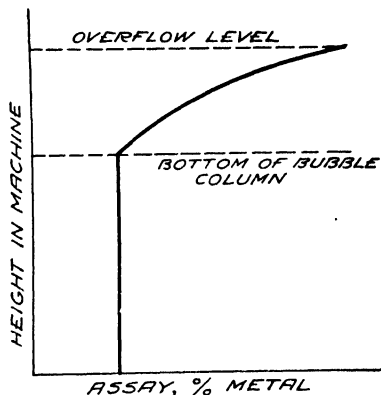


Fig. 9

first be accustomed. This is most easily done by choosing a relatively quiescent part of the column and looking at it with the sole purpose of resolving the coarser individuals. After this end is attained it is easy to distinguish different minerals and to follow their paths.) All solid particles are falling; at a rate that seems enormous due to magnification. But this difference is apparent: That the particles of non-floatable mineral tend to follow

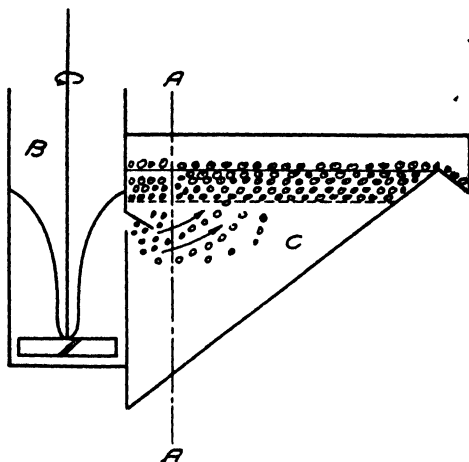


FIG. 10

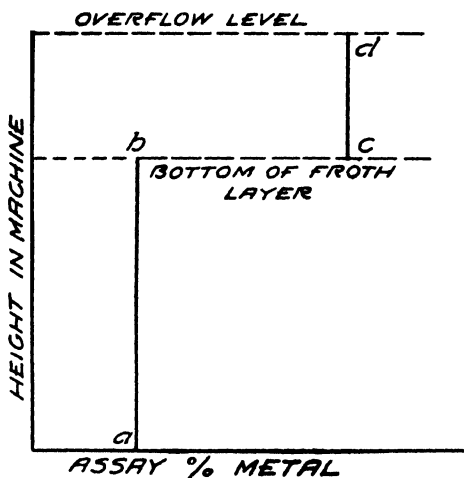


FIG. 11

the middle of the stream and—significantly—having run over the upper surface of a bubble, they drop directly to the upper surface of the next lower bubble in their path, while the particles of floatable mineral do not drop off at the horizontal equator of the bubbles but follow around on the under side and cling sufficiently so that several may collect temporarily in a little patch at the bottom pole. This fact alone shows that the floatable particles are somewhat retarded in their fall relatively to the non-floatable particles, by some attractive force exerted on them by the bubbles. The nature of this force is indicated by Exp. 4 to be the surface tension of the interface between water and an oil sheath surrounding the bubble.

Its effect is to slow down the falling rate of the floatable particles relatively to the non-floatable. By regulating the bubble supply so that the mean rising rate of bubbles in the column is intermediate between the mean falling rates of floatable and non-floatable particles, the former have a resultant rise and the latter a resultant fall, and this effects separation.

The method of bringing about attachment of floatable particles to bubbles in the agitation process and the manner of that attachment both differ from the bubble-column process. So far as it is possible to determine, lacking ability to see into the mechanism as can be done in the latter operation, attachment in the agitation process is brought about by precipitating gas from solution preferentially at the surfaces of properly prepared particles of floatable mineral, and the mineral particles on the bubbles are at the air-liquid rather than at a liquid-liquid interface. The agitation method thus becomes one of the group of pulp-body processes, which includes the boiling, chemical-generation, and vacuum processes, already mentioned. These latter can be peered into with a glass and thus supply grounds for inference as to the mechanism of the first.

26. Boiling process. If a sandy pulp—48-mesh, deslimed with suitable collecting and frothing agents is boiled, the floatable mineral collects on the surface of the boiling pulp as a froth. If water containing individual particles of floatable mineral coated with a collecting agent is slowly heated under microscopic observation, bubbles may be seen to form on the particles and grow, some to a size that will lift the particles. Since no bubbles are present at the beginning nor any introduced externally during the operation, those that appear must have come by precipitation from solution in the water.

Sheet platinum may be substituted for the individual floatable particles for microscopic investigation. It has the advantage that it may be cleaned by acid and basic solutions without the danger of reaction, and may be burned for further and final cleaning.

Bubbles do not precipitate on cleaned platinum. Nor on uncontaminated gangue particles. Nor on platinum so heavily coated with oil that the oil constitutes a visible liquid layer.

The froth in the boiling process looks like clotted aggregates or floccules of mineral particles bound by small bubbles. It clearly forms near the bottom of the pulp and rises to the top without change.

27. Vacuum process. If a similar sandy pulp similarly prepared is subjected to a vacuum, the behavior is entirely similar to that described in Exp. 26, as is also the behavior of larger individuals observed microscopically. The conclusion is, therefore, again that the concentration is completed in the body of pulp by precipitation of gas on the floatable-mineral particles.

28. Chemical-generation process. If a similar ore containing calcium or iron carbonate, similarly suitably prepared, is treated with dilute acid solution, the behavior is precisely similar to that described in Exps. 26 and 27, as is also the behavior of individuals. This is also, therefore, the same kind of a process.

The agitation process makes a froth of the same nature as is made in the other pulp-body processes; concentration takes place in the pulp body; it is primarily inferential that the mechanism of air attachment is likewise the same, i.e., a matter of precipitation. Direct evidence on this score follows:

29. Pressure measurements made by means of an opening drilled in the back of one of the impeller blades in an agitation machine showed that, at operating speeds, there was a **gauge vacuum** of 10 to 12 in. of mercury. The actual vacuum was higher, of course, but was relieved by water vapor and air that distilled into the gas space between the pulp at the impeller blade and the gauge.

In an agitation machine sealed to prevent vortex formation over the impeller, a cloud of gas bubbles appeared around the impeller at flotation speeds. With introduction of external air prevented, this air can only have come from solution.

30. A flotation test was made in which external air was introduced in very fine bubbles into a pulp stirred at different speeds. No flotation occurred with the stirring below a speed that recorded pressure drop on the gage. Flotation started at the speed at which pressure drop started, whether external air was introduced or not, and increased in rapidity and extent with increase in speed of rotation.

The agitation process is, therefore, a continuous vacuum process that may be operated at atmospheric pressures. As fast as the air content of the pulp is diminished by precipitation it is replenished by solution of finely divided air taken in by the vortical action of the rapidly revolving stirrer, beaten into fine bubbles by the moving blades, and subjected to super-atmospheric pressure in the zones in front of the blades.

The minerals at the bubble surfaces are probably in the gas-water interface rather than in the oil-water interface, as has been inferred to be the fact in the bubble-column process. The particles are more strongly held, as is to be expected from the higher surface tension of the air-liquid interface. Precipitation must start as a single molecular individual, and assuming a monomolecular oriented film of contaminant on the particle, this individual must be in contact with this film, without any intervention of an oily bubble sheath. The final bubble consists of a number of these individuals added to the first, hence the mass of gas constituting the bubble filling must be in contact with the contaminated particle surface, which is to say that the particle is in the gas-water interface.

The observations on gas precipitation would seem to be rationalized by the following hypothesis, viz.: That such precipitation is effected by reason of a local rise in vapor tension at a solid-liquid interface in a system in unstable equilibrium with respect to vapor tension.

If we consider the system consisting of water saturated with air at atmospheric pressure and the temperature prevailing, and a sheet of platinum with one-half contaminated and one-half clean surface immersed therein, all in a vacuum cell, then as vacuum is applied, vapor-pressure equilibrium between the liquid and its surroundings is upset in such a direction that water must vaporize and dissolved gases must pass out of the liquid, in order to restore equilibrium. Vaporization and gas evolution, under such circumstances, will take place first from those liquid surfaces where vapor tension is highest, which is to say—all other things being equal, where surface tension is least. Disregarding for the moment the walls of the container, there are three surface tensions to consider, viz.: the liquid-gas or upper surface, the liquid-clean platinum interface, and the liquid-contaminated platinum interface.

As to the surface tension of the liquid-clean metal interface, reasoning by analogy only is possible, but such reasoning points toward the conclusion that this tension is higher than the liquid-gas interfacial tension. For we know that in the case of immiscible liquids of different surface tensions toward air, the liquid-liquid tensions lie between the respective two liquid-air tensions. For

~~example~~, the surface tension¹⁰ of mercury against air is 513 dynes per cm., of ~~water~~ against air 75 dynes, and of carbon bisulphide against air is 30 dynes. The ~~interfacial~~ tension of water against mercury is 392 dynes per cm.; of ~~carbon bisulphide~~ against mercury, 387 dynes; of water against carbon bisulphide, 42 dynes per cm. The surface tension of platinum against air at the ~~solidifying~~ point¹⁰ is 1690 dynes per cm. Assuming that the surface tension of ~~solid platinum~~ against air is not less than this figure and that the ~~interfacial~~ tension ~~water-platinum~~ lies between 75 and 1690 dynes per cm., in analogy to ~~water-mercury~~, the vapor pressure at this interface of elevated surface tension will be lower than that of the free gas surface and consequently vaporization ~~should take place~~ at the free gas surface and not at the clean metal surface.

On the other hand, and by the same method of reasoning, the surface tension of the contaminated platinum contact with water is to be expected to be lower than that of the free water surface. For the surface tension of an oil-water contact is lower than that of an uncontaminated water-gas contact. The vapor pressure, rising with fall in surface tension, should be higher at the contaminated metal surface than at the gas surface, and water is to be expected to vaporize there.

Unless the surface is plane, vaporization will start at and be concentrated at the point inequalities (local points of least surface tension and highest vapor pressure due to geometrical configuration). Dissolved air will vaporize rapidly into these incipient bubbles on account of the initial zero partial pressures of air therein, so that the rapid growth of bubbles from mere pin-point size to sensible dimensions, which is the observed behavior, is the expectable result.

The fact that when a solid is coated with a thick layer of oil, so heavy as to be observable as a liquid body, gas precipitation does not take place, detracts in no way from the hypothesis offered. At such a liquid-liquid interface there is no reason for gas molecules to collect as a bubble. Those that leave the water, as individuals, because of high vapor tension, pass, as individuals, into the liquid oil, or *vice-versa*, and partition takes place until equilibrium is established.

The oil-platinum interface is, according to hypothesis, a region of high surface tension and correspondingly low vapor tension, so precipitation of gas that passes from the water into the oil is not to be expected at the oil-platinum interface.

Flotation Machines

Flotation machines are to be classified on the basis of the method used for introducing the gas for buoying the floatable mineral. Two principal types of machines and a class that is a hybrid of the two prevail today. Fifty, however, would not number the species—usually named for their inventors—included in these three genera.

Bubble-column machines. The most widely used and most efficient of the bubble-column genus are the pneumatic machines, of which the apparatus of

¹⁰ "Smithsonian Physical Tables," 146 (1916).

Fig. 1 is typical (Callow). Repetition of treatment is obtained by making the porous-bottomed trough long, with or without subdivision. With coarse feed pulps that settle on the blanket a V-shaped trough is used with a canvas covered air-supply pipe rotating in the bottom (MacIntosh). Rotation sloughs off the settled sand. When pulps containing soluble iron are made alkaline with lime, a mixture of iron carbonate and calcium carbonate and sulphate precipitates in the blanket pores and increases resistance to air flow, with consequent power increase. For such pulps a form of pneumatic machine is used with air introduced through open pipes. In this case enough air must be used and the trough must be so designed as to encompass rapid turbulent circulation of the pulp in order to break up the large masses of air entering at the pipe terminals into small bubbles, and provision must also be made at some point in the circulatory flow for substantial quiescence of the pulp for a sufficient time to permit the finely divided impregnating air to rise and form a bubble column (Forrester, Welsch, Hunt).

Another form of bubble-column machine (Cascade) effects air introduction by discharging a stream or jet of pulp into a body of pulp. The jet breaks up into small masses and drops, each of which acts on entering the body of pulp as a solid particle and drives a bubble of air ahead of it into the mass. The action is familiarly exemplified by the jet from a faucet entering a wash bowl.

Agitation-froth machines. The best-known machines of this class are of the type of Fig. 10 (Minerals Separation). Repetition of treatment is obtained by building a number of units side-by-side with common walls and flowing the discharge from one settling chamber into the bottom of the adjacent following agitating compartment.

Hybrid machines. The sub-aeration type has a cross-section similar to that of Fig. 10. Additional air is introduced through a hollow agitator shaft and a specially shaped impeller (Ruth, Groch, Fahrenwald), or through a pipe entering the agitating compartment below the pulp-level therein (Stenning, Owen, Hebbard, Kraut).

Some agitation machines cascade the pulp from the agitating chamber into the settling box (Janney); others introduce air through a porous medium located in the bottom of the settling compartment (Janney, Jones-Belmont).

Differential Flotation

Differential flotation is the operation of floating one mineral selectively in a pulp that contains two or more minerals of the class ordinarily considered floatable, e.g., galena is floated while sphalerite and pyrite remain submerged; chalcocite and chalcopyrite may be floated away from pyrite; sphalerite, from pyrite and pyrrhotite.

Experimental

31. If a pulp containing galena, sphalerite and pyrite and the usual gangue minerals, made alkaline (pH 8.5 to 10) with lime or sodium carbonate, is digested at normal temperature for a short period (5 to 30 min.) with alkali cyanide, present in the proportions of 0.5 to 2 or 3 parts per ton of ore (and 1 to 3 tons of water), and then floated with 0.05 to 0.1 lb.

potassium ethyl xanthate and an equal amount of pine oil, the galena floats preferentially. Further digestion of the residue with copper sulphate (1.5 to 2 lb. per ton of ore) present and re-flotation—with more xanthate and pine oil, if necessary—raises the sphalerite. The iron may finally be floated by intensification of the flotation effect, as by the addition of more xanthate and frothing oil, or by addition of 1 or 2 lb. per ton of ore of a collecting oil such as coal- or wood-tar creosote, with or without acidification of the pulp.

Most of the chemical reactions involved in this operation have already been discussed. Zinc cyanide formed on the sphalerite surface prevents formation of zinc xanthate (Exp. 19). Pyrite probably becomes coated with insoluble ferro- and ferricyanides. Galena is not attacked, and thus reacts with xanthate. Copper sulphate subsequently removes the cyanide coating from the sphalerite and coats it with copper sulphide (Exp. 19), which, with the xanthate, furnishes the sphalerite with a water-repellent coating. The action of the iron at this point is not known. Microscopic examination of pyrite in the tailing from zinc flotation shows the surface considerably altered, but still exhibiting patches of pyritic luster. It may be that it is these portions of the surface that become coated with collecting oil or further xanthate, finally resulting in flotation.

Brownian Movement. Microscopic examination of the pulps and products of Exp. 31 reveals varying degrees of Brownian movement. Controlled experiments with the various solid substances separately immersed in solutions of the various reagents, shows that Brownian movement of the solid in given solutions is a distinctive matter, and that direct correlation exists between movement and floatability.

Experimental

32. Galena, sphalerite, pyrite and quartz, dry powdered through 200-mesh (0.074-mm.) screen, were suspended individually in distilled water, the proportion of solid to liquid being 1 to 4 by weight. After allowing a short time for sedimentation, the supernatant cloudy liquids were examined separately by dark-field illumination. Quartz was in motion, the other minerals at rest. Sodium carbonate was next added to all samples in an amount equivalent to 1.5 to 2 lb. per ton on the solid. All four minerals were in vigorous Brownian movement. Potassium cyanide was added next, in an amount equivalent to 1 lb. per ton of solid; the motion of quartz and galena was unaffected, that of sphalerite and pyrite was intensified. Potassium xanthate added to the preceding solutions in an amount equivalent to 0.1 lb. per ton of solid stopped the motion of the galena but did not affect that of the other minerals. Pine oil in the same proportion, added additionally, caused no further change. Reference to Exp. 31 shows that galena will float at this point. Further addition of copper sulphate did not affect galena, pyrite or quartz, but did stop the motion of sphalerite. Exp. 31 demonstrates that sphalerite will float at this point.

It thus appears that when mineral particles are in Brownian movement, even though suitable collecting and frothing agents are present in proper amounts, they will not float; and that the converse is also true.

If the classical theory, which ascribes Brownian movement to reaction of suitably sized particles to the impulses of bombarding molecules, is accepted, there is neither rhyme nor reason to the phenomena described. It is true that flocculation was more noticeable when particles were quiescent than when they were in motion, but on the other hand, in pulps in motion, there were

moving floccules much larger than many of the individuals and floccules at rest in quiescent pulps. Even more conclusively against the molecular bombardment theory, however, is the following experiment.

33. Quartz of high purity was broken into lumps of about 1/8- to 3/8-in. size, soaked for several days in chrome acid cleaning solution, washed for several weeks in running tap water, further washed ten or a dozen times by decantation with distilled water, then dried and ground in a porcelain mill to pass 200-mesh. The powder was next ignited for an hour at 1000°C. This powder was in vigorous movement in m/1000 sodium carbonate solution and at rest in m/1000 sulphuric acid.

A slide was made of the suspension in carbonate solution, the cover glass sealed on with paraffin wax, leaving portions of the periphery toward opposite ends of the slide unsealed. The quartz was in active movement. A drop of m/500 sulphuric acid was then placed at each of the unsealed places. The progress of the diffusing acid could be followed across the the slide, using a large-field low-power objective (16-mm.), as a wave front of cessation of movement. When the wave fronts from the two ends met near the center all motion on the slide had ceased.

It is to be borne in mind particularly at this point that the slide had been prepared of such dilution that individual particles were many diameters apart and that the largest were at least 10-times the diameter of the smallest. The behavior of individuals was observed at 2500-X. In ceasing to move they first slowed, seemed to settle, struck bottom (the slide), gave one or two spasmodic wriggles, perhaps even went into suspension again for a few moments, but finally came to rest on the bottom, as individual particles, not parts of a floc.

When all the particles had come to rest the experiment was continued by adding m/500 sodium carbonate solution as the sulphuric acid solution had previously been added. Now a wave-front of resumed Brownian movement passed across the slide until finally the whole field was again in motion, perhaps more sluggish than in the first instance, but nevertheless unmistakable.

The same process of stopping and revivification has been performed in a test-tube, its progress followed by slides prepared at intervals.

It would seem that these experiments throw serious doubt on any hypothesis that ascribes suspension of such particles as these, and their irregular vibratory motion, to direct bombardment by the molecules of the suspending liquid. Particles of a given size were stopped and started again in Exp. 33 with no visible change in their size or state of aggregation, and consequently with no visible change in the conditions which, mechanically, would enable them to resist the effects of impact. Adsorption of a microscopically invisible layer of something in the sulphuric acid solution, with consequent increase of mass, would seem to be disproved by the fact that the range in size of particles in motion both before and after the sulphuric acid treatment was easily ten times the diameter of the smallest moving particle, and adsorption, by whatever mechanism, amounting to a thousandfold increase in mass of any of the particles is inconceivable. Yet increase to a yet greater extent in the mass of the smallest particles is necessary to explain their stoppage on mechanical grounds.

Change in momentum of the bombarding particles would seem to be barred on the principle of equipartition of energy. If, as this principle states,

each of the particles in motion in such a system as we are dealing with has, when the temperature is uniform throughout, the same amount of energy, then changes in association of the molecules of the suspending medium, ion hydration, introduction of new ions and molecules, and the like, will not change the force of the bombardment.

It would seem, further, that attribution of Brownian movement in these pulps to a molecular or ionic bombardment, greater either in its intensity or effectiveness when sodium carbonate is present, than when sulphuric acid is present, is completely upset by the fact that if the chemical nature of the suspended particles is different, if for instance, calcite replaces quartz or galena or sphalerite, sodium carbonate does not produce movement. It is true that the converse cannot be said, viz: that sulphuric acid does produce motion with calcite. But by changing again the nature of the suspended material, this time to a highly silicious volcanic tuff, we have a mineral that moves in the presence of sulphuric acid.

A yet more informative correlation exists between Brownian movement and the chemical nature of the particle surfaces, than has been shown between motion and floatability. When the particle surfaces are coated with a readily ionizable salt whose solubility in the suspending medium lies somewhere between a fraction of a milligram and 20 to 30 mg. per liter, then the particles, if suitably small, move. With surface solubilities outside this restricted range, the same particles are at rest.

34. Galena is at rest in m/1000 solutions of H_2SO_4 , K_2SO_4 , $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$, $MnSO_4$, $MgSO_4$, $KHSO_4$, $FeSO_4$, Ag_2SO_4 (solubility $PbSO_4 = 42$ mg. per l.); HCl , KCl , $CaCl_2$ (sol. $PbCl_2$, 6730 mg. per l.); HNO_3 (sol. $Pb(NO_3)_2$, 390 000 mg. per l.); K_2CrO_4 (sol. $PbCrO_4$, 0.2 mg. per l.); $S:C (OC_2H_5)_2 SK$, (sol. $[S:C (OC_2H_5)_2]_2 Pb$, 0.2 mg. per l.). Galena is in vigorous motion in m/1000 Na_2CO_3 (sol. $PbCO_3$, 20 mg. per l.), and in more sluggish motion in $K_4Fe(CN)_6$ (sol. $Pb[Fe(CN)_6]_2$, "insoluble").

Precipitated lead carbonate, made by reacting lead nitrate with sodium bicarbonate and washing the precipitate 6 times with 50 to 100 volumes of distilled water per wash, then filtering and drying at 100-110°C, then grinding in agate, is at rest in distilled water and in m/1000 alkali sulphate, iodide (sol. PbI_2 , 440 mg. per l.) and chloride solution; it is in vigorous movement in m/1000 solutions of Na_2CO_3 , $NaHCO_3$ and $(NH_4)_2CO_3$, $NaOH$ (sol. $Pb(OH)_2$, "sl.s"), KIO_3 (sol. $Pb(IO_3)_2$, 12 mg. per l.), K_2CrO_4 , K_2HASO_4 (sol. $PbHASO_4$, "i"), $Na_2C_2O_4$ (sol. PbC_2O_4 , 1.6 mg. per l.), Na_2HPO_4 , $K_2Cr_2O_7$ (probably forms the chromate).

Precipitated lead chromate acts similarly to the carbonate.

Precipitated lead iodate moves in m/1000 Na_2CO_3 .

Precipitated lead sulphate does not move in distilled water, nor in m/1000 solutions of K_2CrO_4 , Na_2CO_3 , $NaOH$, or $NaHCO_3$.

Sphalerite is at rest in the same solutions as those listed in which galena is at rest. The solubilities of the sulphate, chloride, and nitrate of zinc are higher than those of lead; the solubility of zinc xanthate is about 330 mg. per l.

Sphalerite moves in m/1000 Na_2CO_3 (sol. $ZnCO_3$, 10 mg. per l.), $NaCN$ (sol. $Zn(CN)_2$, "i"), $NaOH$ (sol. $Zn(OH)_2$, 4.2 mg. per l.), and $K_4Fe(CN)_6$ (sol. $Zn[Fe(CN)_6]_2$, "i").

Quartz is at rest in m/1000 H_2SO_4 , $Al_2(SO_4)_3$, $Fe_2(SO_4)_3$, HCl ; HNO_3 . It exhibits vigorous motion in distilled water and m/1000 Na_2CO_3 and $NaOH$. It has appreciable motion in m/1000 $ZnSO_4$, $MnSO_4$, $MgSO_4$; KNO_3 and feeble motion in $CuSO_4$, $KHSO_4$, $CaCl_2$ and KCl .

A kinetic picture that, lacking all direct proof, yet corresponds to the observed facts, is that particles in Brownian movement are, for all practical purposes, visible ions,—inert masses, so far as their visible portion is concerned, but having anchored in the surface of their lattice structure, in greater or less number, one of the ions of a compound that is formed by reaction of the original particle-surface compound with one of the constituents of the suspending medium. The other ion of this reaction product may be pictured as diffusing throughout the suspending medium, subject to the forces that cause diffusion, and, by inter-ionic attraction, transferring the effect of these forces to the visible ion.

If it is contended that diffusion itself is due to molecular activity (bombardment), and that the present picture, therefore, differs only in detail from the classical presentment, the author has no quarrel with such a stand. But from the viewpoint of interpretation of flotation phenomena the difference in detail is vital. For it forces us to regard each and every particle in a flotation pulp as a potential participant in one or more chemical reactions which fundamentally determine the behavior of the particles in the flotation operation. And it gives us a key to control. If we would float any particle we must cause it to react with some added ingredient to form a compound substantially insoluble and non-ionizable in the suspending medium, and having, furthermore, such surface characteristics that the energy of the interface between it and air or a frothing oil is less than that between it and water.

If we would prevent a particle from floating, several courses are open to us. (1) We may use a collector that does not react with the particle or with any reaction product of the particle with any of the other ingredients of its environment. (2) We may use a collector that, although it reacts with one of the ions of the particle, does not form therewith a substantially insoluble compound (e.g., potassium ethyl xanthate with sphalerite). (3) We may add, prior to the introduction of the collector, a substance that reacts with the particle surface to form a compound less soluble than the reaction product of the collector with the unaltered particle.

Further applying the same principles we may float a particle not normally floatable with a given collector by first adding a reagent that will react with the particle surface to deposit there, in the form of a relatively insoluble yet slightly ionizable compound, an ion which forms with the collector a less soluble or less ionizable compound that is water-repellent. (Copper sulphate with sphalerite and xanthate; lead or copper salts with calcite and xanthates.)

Logical extension of the principles here laid down leads to the conclusion that flotation or depression of any mineral involves solely investigation of its reaction possibilities and of the properties of its reaction products. Ordinary lists of chemical compounds and their solubilities will be extremely helpful in such an investigation, but failure to find publication of a given salt is not to be accepted as conclusive evidence that such a salt may not be formed;

characterization in a table as "insoluble" does not necessarily mean insolubility in the extremely low concentrations of the flotation cell; and finally a solubility figure based on ordinary methods of determination is not necessarily the figure that will obtain when precipitation or non-precipitation at a mineral-particle surface is involved. The only certain test of reaction and precipitation is an abstraction test made with the mineral itself, under conditions of reagent and hydrogen-ion concentrations, ultimately, that approach those in the flotation cell.

Direct tests on the water-repellent character of coatings may be made in the apparatus described by Taggart, Taylor and Ince.¹ When suitable mineral particles are not available, actual testing in a flotation machine must be resorted to, but conclusions in this case are necessarily a matter of inference.

OPTICAL SENSITIZATION IN PHOTOGRAPHY*

BY WILDER D. BANCROFT, J. W. ACKERMAN AND CATHARINE A. GALLAGHER

In 1873 Vogel¹ published a short paper on the sensitization of silver bromide plates by certain dyes. "I obtained from England some silver bromide dry plates prepared commercially by Wortley, who used a process some details of which are kept secret. I exposed these to the spectrum and found to my surprise that they were more sensitive in the green to the E line than in the blue to the F line. The sensitivity, contrary to all previous experience, was greater for light which usually is rather inactive chemically than for light which is usually very powerful chemically. This phenomenon caused me to study more carefully the sensitivity of silver bromide to spectrum colors.

"I experimented with silver bromide in two forms: (1) as a so-called wet plate, moist with the silver nitrate solution from the bath in which the plates were sensitized; (2) as a dry plate, prepared by washing off the silver solution and drying. The dry silver bromide showed a much greater color sensitiveness than the silver bromide under the silver solution. With an acid developer the sensitivity of the wet plate ended about half-way between D and E, practically in the middle of the yellow. The dry plate was sensitive two millimeters beyond the D line, into the orange.

"The behavior of the two plates was qualitatively very different. With the moist silver bromide there was a very strong action between G and F (in the indigo and blue); at F it decreased very rapidly and there was only a slight fogging beyond E. With the dry silver bromide the action in the blue was much weaker than with the wet plate; but the decrease was much less in the longer wave-lengths and the effect extended beyond D, as has been said. Dry silver bromide is therefore more sensitive for the less refracted wave-lengths and the wet plate for the more refractive, the blue, rays of the visible spectrum.

"Silver nitrate is a powerful sensitizer for ordinary photographic plates and increases the sensitivity because it combines chemically with the iodine or bromine set free during the exposure. The reason why this effect takes place chiefly in the blue is because the blue rays are absorbed more strongly by the film than the others.

"This explanation for the action of silver nitrate on silver bromide made me suspect that the English silver bromide plates must contain a substance which absorbs green more strongly than blue. Dry plates are often covered with all sorts of substances, such as tannin, gallic acid, caffeine, and morphine,

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¹ Ber., 6, 1302 (1873).

all substances which combine with iodine and bromine and which therefore sensitize. Occasionally people add a yellow dye to cut down the amount of the chemically active blue light. Practically nothing is known about the optical behavior of these 'preservatives,' and there is no certainty that they are beneficial.

"The Wortley plates contain uranium nitrate, rubber, gallic acid, and a yellow dye as a covering. To determine whether this covering had an effect, I washed a Wortley plate with alcohol and water, obtaining thereby a plate with no increased sensitivity in the green. I then attempted to impregnate silver bromide with a substance which would absorb the yellow rays chiefly and would combine with free iodine or bromine, in the hope of increasing the sensitivity in the yellow. I selected corallin which Professor Liebermann most kindly placed at my disposal. A very dilute solution showed an absorption band between D and E; in more concentrated solutions the absorption extended beyond D; on the other it was quite transparent to the blue at F.

"I dissolved corallin in alcohol and added enough of it to my bromide collodion to color the latter a strong red. With this collodion I prepared silver bromide dry plates which were colored distinctly red. On exposure to the spectrum, my prediction was verified. The sensitivity of the plates was marked in the indigo and decreased towards the blue, being quite weak at F. From there on the sensitivity increased, becoming almost as great in the yellow as in the indigo. A method had therefore been found for making silver bromide plates as sensitive for yellow, a hitherto chemically inactive color, as for indigo which had been considered the most active color chemically.

"After these experiments I believed that a dye, which absorbed red strongly and which reacted with bromine, would increase the sensitivity of silver bromide for red. I found such a substance among the green aniline dyes. It absorbed strongly the red rays in the middle between D and C. With higher concentrations the absorption extended further towards D; but yellow, green and blue were transmitted with very little loss. A collodion dyed with this green was actually light-sensitive into the red. The sensitivity decreased from indigo to yellow and then increased, there being a strong effect in the red where the absorption band occurred.

"From these experiments I believe myself justified in concluding that we are able to make silver bromide light-sensitive to any color one pleases, or to increase the existing sensitivity for any particular color. It is only necessary to add a substance which will promote the chemical decomposition of the silver bromide and which absorbs the color in question without absorbing the other colors appreciably. Perhaps we may be able some day to photograph infra-red as we now photograph the ultra-violet.

"It should be possible also to overcome the disturbing effect due to the photographic inertness of certain colors. The following experiment shows what can already be done in this line. I photographed a blue band on a yellow ground. With an ordinary silver iodide collodion plate I obtained a white band on a black ground. With a silver bromide corallin plate, on which blue and yellow are equally effective, I obtained of course no contrast. I then put

before the objective a yellow glass which cut off most of the blue and let the yellow pass practically unchanged. After a sufficiently long exposure I obtained a dark band on a light ground."

We have only to consider the panchromatic films, the orthochromatic films, and the films for aerial photography which are sensitive in the extreme red, to see how accurate Vogel's forecast was.

Carey Lea¹ did not believe in Vogel's conclusions. Describing his work, he said: "It seemed therefore a matter of interest to determine whether any general law existed that when a metallic compound capable of reduction by light was placed in contact with a body capable of being oxidized (or of uniting with chlorine, bromine or iodine) the capacity of the metallic compound by any particular portion of the spectrum would be influenced by the color of the body placed in contact with it. If, for instance, a ferric salt be placed in contact with an oxidizable body of well-marked color, will the reducibility of the ferric salt by particular rays be modified? And if so, will it follow the law announced by Dr. Vogel for silver bromide? To solve this question, I have made an extended series of experiments. But I have not been able to verify the existence of such a law.

Ferric Salts

"Ammonia ferric oxalate was selected as the most easily reducible of the ferric salts.

"Strips of paper were first thoroughly colored with aurine, with aniline blue and aniline green; they were then impregnated with the ferric salt, and were exposed to light side by side with ordinary white paper similarly impregnated with the ferric salt.

"The exposure of these and of all the following preparations was managed in the following manner. Colored glass was obtained of shades corresponding as nearly as possible with the colors of the spectrum. Violet and green glass could be found in commerce of suitable shades. The other colors were obtained by dissolving suitable transparent pigments in varnish and coating glass with it. With the aid of aniline colors and colorless varnish, the most brilliant shades were obtained, with a perfect transparency. These glasses were next cut into strips ten inches long and five-eighths wide and arranged to form a sort of artificial spectrum, under which papers of different preparation could be simultaneously exposed. It is evident that in some respects this mode of operating is less advantageous than that of exposure to a real spectrum. But the disadvantage is compensated by the possibility afforded of a most accurate comparison of the effects of the various substances, inasmuch as the different papers can all be exposed simultaneously and all receive precisely the same impression. There results an accuracy of comparison which can perhaps be obtained in no other way.

"The papers prepared with ferric salt alone, and also those with ferric salt in contact with the colors named, were simultaneously exposed. They

¹ Am. J. Sci., (3) 7, 200 (1874); 9, 355 (1875); 11, 459 (1876).

were then plunged into solution of ferricyanide of potassium, which renders evident whatever reduction has taken place by the production of Turnbull's blue, the unreduced portions remaining white.

Result.—"The series of experiments was carefully repeated three times. The aniline blue was found to be entirely without influence; the printed spectrum obtained corresponded in every respect with that of the plain ferric salt. The aniline green slightly diminished the impressibility, but not more in one part than another. Aurine produced this effect still more strongly.

"Neither coloring matter exerted any specific influence on the impressibility by any particular portion of the spectrum.

"Other coloring matters were tried without results of special interest, except that a cold aqueous extract of *safflower* (carthamus) much heightened the sensitiveness to the whole spectrum, perhaps doubling it.

Silver Chloride

"A number of experiments led to the following results: Coralline increased the sensitiveness to all the rays, but especially to blue and violet, in which the increase is very considerable.

"Rosaniline increased the sensitiveness to blue and violet, but diminished all the rest.

"Aniline blue diminished sensitiveness to green, increased it to yellow, and was without effect on the rest.

"Aurine diminished sensitiveness to all.

"Mauveine and aniline green were without effect.

"Litmus reddened by acetic acid strongly increased the sensitiveness to the blue and violet, and somewhat diminished it to the red and orange.

"Here we have three red colors increasing the sensitiveness to the blue and the violet. But one, coralline, increases the sensitiveness at the red end also, whereas red litmus and rosaniline diminish the sensitiveness at the red end.

Silver Iodide

"Silver iodide papers, imbued with various coloring matters and containing free silver nitrate, were exposed to the different rays with the following results:

"*Red and Orange rays.* None of the coloring matters tried increased the sensitiveness to these rays.

"*Yellow rays.* Aniline blue and green increased the sensitiveness to these rays somewhat, mauveine perhaps a very little. Coralline diminished the sensitiveness a little, aurine and rosaniline a good deal.

"*Green rays.* The aniline green (a bluish green) increased the sensitiveness to the green ray somewhat, aniline blue (a violet blue) increased it a very little. Mauveine was without influence, whilst coralline, aurine and rosaniline gave weaker results than the plain iodide paper, the last two much weaker.

"*Blue rays.* Aniline green is here again the strongest. Blue and mauveine increased the sensitiveness to the blue rays a little, coralline was without effect, aurine and rosaniline diminished the sensitiveness.

"*Violet rays.* Aniline blue, green and mauveine all considerably increased the sensitiveness, coralline increased it a little, aurine and rosaniline diminished it a little. With ordinary white light the order of sensitiveness was the same as in the violet rays.

"It does not appear that there exists any general law connecting the color of the substance placed in contact with the silver iodide with increased or diminished sensitiveness to particular rays. A violet blue aniline color increased the sensitiveness to the yellow and green rays, but also had a similar effect upon the violet rays. Aniline green increased the sensitiveness to the violet, blue, green and yellow rays, but not to the orange and red; its tendency was to increase the sensitiveness of colors approximating to its own color, whereas coralline increased the sensitiveness to the rays which most differed from its own color.

Silver Bromide

"Silver bromide is at once the most important of all the sensitive substances known, and the most difficult as to the exact determination of its reactions, so much do these vary from very slight causes. Multiplied experiments were consequently made; thirty-five complete spectra were obtained, besides prints from detached portions of the spectrum. Below I give the substances in the order of the greatest sensibility which they conferred, beginning with those that gave the greatest.

Substances which conferred the greatest sensibility to the more refrangible half of the spectrum

Infusion of tea leaves,
Salicine,
Red Litmus,
Coralline,
Aniline blue

Plain bromide,

Aniline green,
Mauveine,
Aurine,
Cold infusion of safflower,
Infusion of capsicum

Substances which conferred the greatest sensibility to the less refrangible half of the spectrum

Salicine,

Plain bromide,

Aniline green,
Mauveine,
Aniline blue,
Aurine,
Infusion of tea leaves,
Coralline,
Infusion of capsicum,
Cold infusion of safflower (carthamus).

"The substances above the 'plain bromide' increased its sensitiveness, those below it diminished it, and in all cases to an extent corresponding with the order or rank in the respective columns.

"Generally speaking, the substances enumerated above exerted very much the same effect on different colors at each end of the spectrum, that is, those that heightened or impaired the sensitiveness to the green acted similarly on

the yellow, orange, and red, and those that heightened or impaired the sensitiveness to the violet rays acted similarly to the blue rays and also to white light.

"The conclusions which I have reached seem to me to establish that there is no general law connecting the color of a substance with the greater or less sensitiveness which it brings to any silver haloid for any particular ray."

While testing a number of red dyes to find a sensitizer for green rays, Carey Lea found that coralline would work but did not consider its action as any function of its color because it exhibited a "still more marked tendency to increase the sensitiveness of silver bromide to the red ray than to the green." He reported that none of the other red dyes used in that experiment had any sensitizing effect.

Obviously, the outcome of Carey Lea's work was a contradiction of Vogel's law and an apparent disproof. He had no explanation to offer in place of that put forth by Vogel, however, and Vogel's law still seems both plausible and possible.

Not much progress has been made since Vogel's time toward solving the mysteries connected with optical sensitizers. The most recent views are involved and complicated to say the least, and must remain largely hypothetical, since they are, as yet, incapable of being tested experimentally. The following quotation is taken from a recent article by S. E. Sheppard¹ of the Eastman Kodak Company research laboratories.

3. Optical Sensitization

"The silver halides are normally photosensitive chiefly in their own absorption region in the blue-violet. Sensitivity to longer waves can, however, be increased by various processes of so-called optical sensitization. The best known of these is the use of certain groups of dyes, which sensitize the silver halide for an extended spectral region which, while not identical with the absorption spectrum of the dye in ordinary solvents, is conditioned by this, and is probably identical with the absorption of dye: silver halide combination.

"In papers by Fajans and Frankenger² on the influence of ionic adsorption on the photochemical decomposition of the silver halides, conceptions were advanced which open up a new view of these optical sensitizing effects.

"They suggested that adsorption of simple cations, as Ag ions, is limited to an electrostatic monatomic layer. The work required, $h\nu$, for transfer of an electron from a bromide ion to an absorbed Ag⁺ ion is less than in the case of the normal surface of the lattice.

"The considerations advanced by Fajans and Frankenger do not, however, seem entirely adequate, for the following reasons. First, the actual surfaces developed in silver bromide crystals are not of the chessboard type described, with alternate Ag⁺ and X⁻ ions. This would be a cubic surface, whereas the faces which are found are dominantly octahedral, i.e., all Ag⁺ ions or all Br⁻ ions. Hence the figure and explanation advanced by Fajans and

¹ Chemical Reviews, 4, No. 4 (1927).

² Z. Electrochemie, 28, 499 (1922); Z. physik. Chem., 105, 255, 373, 329 (1923).

Frankenburger are not realized, except perhaps to some extent for adsorption of Ag^+ ions. But if we consider less an adsorption that an inbuilding of foreign nuclei in the crystal grating, then the quantum changes, i.e., diminution of h necessary for the reaction $\text{Br}^- - \Theta \rightarrow \text{Br}$, etc., becomes in virtue of the deformation of contiguous ions of the crystal lattice. We have here an explanation of the optical sensitizing presented by the very dissimilar substances, viz. metallic silver, silver sulfide, silver iodide, and silver cyanide.

"The writer has indicated elsewhere how this effect may not only lead to anomalous optical sensitizing effects, but also contribute to the concentration of the blue-violet photochemical decomposition about the sensitivity centers, notably of silver sulfide.

"In normal or dye sensitizing there is probably no such inbuilding, but a surface adsorption of the dye. The principal classes of sensitizing dyes are:

- A. Phthaleins, e.g., erythrosin, eosin
- B. Cyanins e.g., carbocyanins, isocyanins

"The first are acid dyes, forming complex anions. These will be adsorbed chiefly by silver ions, and it is known that these dyes do not sensitize well by bathing, but are assisted by the use of soluble silver salts, i.e., by intermediate silver ions. Going to the pronouncedly basic cyanine dyes these form complex cations, and are therefore held by bromide ions. But, such an adsorption involves reciprocal deformations in the bonded ions, so that the displacement of the spectral sensitizing curve is to be expected. Hence, we probably get a superposition of the anomalous and normal optical sensitizing effects. The normal effect follows as an inner photo-electric effect in the dye ion, whereby its reduction potential is raised and silver ions are reduced, which form a latent image about 'sensitivity specks' just as in the case of the photochemical decomposition of $\text{Ag}^+ \text{Br}^-$.

"We may suppose that dyes are either:

- a. Adsorbed to the Ag^+ ions
- b. Adsorbed to the Br^- ions
- c. Adsorbed to homopolar AgBr (or AgI)

Acid dyes, e.g., erythrosin, giving (complex) anions, would be expected to adsorb to Ag^+ ions, and it is a fact that their sensitizing action is supported by free silver ions.

"Basic dyes, e.g., pinachrome, giving complex cations, would be expected to be adsorbed to Br^- ions in the silver halide lattice. It is, however, a noteworthy fact that such dyes are much more soluble (on the alkaline side) in chloroform than in water. Hence, one may anticipate a possible strong adsorption to homopolar AgX pairs, a fact in agreement with the strong adsorption of 'basic' dyes to silver iodide. It is also significant that the sensitizing spectra of these dyes with silver bromide approaches more nearly to the absorption spectrum in chloroform than to that in water or alcohol.

"The exact mechanism of optical sensitizing is not yet clear. The relatively simple cases of sensitization by mercury atoms excited to the resonance

potential and producing active hydrogen by radiationless collisions have been adduced as significant for photographic sensitizing with the dyes. We have, however, in the dye:silver halide complex, a very complicated system, in which we can scarcely picture activated dye molecules, as wholes, colliding with the silver salt. We must rather conceive of a number of changes being excited by absorption of light, ranging from a transmission from non-polar linkages (as in photography) to reversible electron transfers (reduction and oxidation) and finally to non-reversible changes (hydrogenation and dehydrogenation). If this series is borne in mind, representable by three energy levels of disturbance of the dye:silver halide system, then the radiation antagonism in dye sensitizing and desensitizing effects appears as a combination of the true (virtual) photo-chemical equilibria with pseudo-antagonistic reactions (destruction of photo-product by certain radiations, as in photocatalyzed auto-oxidations) and with secondary topochemical effects (Lüppo-Cramer's nucleus isolation)."

In a later discussion¹ of the mechanism of sensitizing Sheppard says:

"Provisionally, the mechanism of optical sensitizing, on the basis of adsorption is as follows: Supposing that the colored dye cation is electrostatically held to bromide ion, but that this original electrostriction passes into homopolar combination, in agreement with the conclusion that the colored form is notably less polar than the colorless, on absorption of light by this in its own absorption region an electron is freed, possibly from the bromide ion, and a silver ion reduced, or indirectly by the 'reduced' dye cation.

"This mechanism would give only one Ag atom for each dye molecule adsorbed to the silver halide. Recently Leszynski has published evidence that (with erythrosin) up to 20 silver atoms may be photochemically reduced per dye molecule acting as sensitizer. He suggests that the photoelectron may travel some distance through the silver halide crystal, and effect a chain reaction of rather high efficiency, or that the reduced silver continues to act as an optical sensitizer.

"As an alternative to this, it may be suggested that in the photodecomposition of adsorbed dye on silver bromide, the dye molecule is practically exploded with release both of several free electrons, and also of very active free radicals. The photochemical efficiency might then be considerably greater than unity, but would be a pronounced function of the *intensity* of the illumination.

"That the optical sensitizing is connected with the photodecomposition of the dye is supported by the fact that the addition of silver ions to aqueous solutions of the dye greatly accelerates its decomposition (bleaching) by light. Our experiments on this indicated that below a molar ratio of about 1.5 Ag⁺ to 1 mole dye, little or no acceleration of decomposition was produced, while from this point the acceleration was approximately proportional to the silver concentration. Whether the apparent threshold is significant or not has not yet been determined."

¹ J. Phys. Chem., 32, 751 (1928).

Two years later Sheppard wrote:¹

Optical Sensitizing

"In gaseous systems an atom of a gas such as mercury, absorbing in its series spectrum, or a molecule of chlorine absorbing in its band spectrum, becomes excited. This excited molecule can transfer the absorbed energy in a single act to another molecule, by a type of inelastic collision, whereby the assaulted molecule is decomposed. It is still an open question whether the optical sensitizing of silver halides by colloid metals, colloid silver sulfide, and by dyes is due to excitation and rayless collisions or to another process, perhaps a photoelectric effect. It appears to be a condition of optical sensitizing by colloid silver and the like that the sensitizer be sub-divided to amicroscopic particles, containing very few atoms, and perhaps monatomic in one dimension. Fajans and his collaborators have found that silver halide with silver ions adsorbed is sensitized for visible decomposition by longer waves, while adsorbed thallous ions sensitized both for visible and latent image formation. He attributes this to a deforming action of the surface cations on adjacent halide ions, the deformation being greater than in the symmetrically arranged interior of the crystal. The deformed halide ions are supposed to lose electrons for a lower energy quantum. It is also possible that it is due to a reduction of the electrostatic energy by lattice loosening. Fajans has applied the deformation concept to the case of colloid silver sensitizing, assuming deformation by adsorbed silver. Since there is no quantitative evidence on the adsorption of silver, the application remains hypothesis.

"It is also possible that the sensitizing is due to a photoelectric electron emission from the silver, below its critical threshold at about 3300 Å., equivalent to 3.6 volts. This appears reasonable for metal particles in a salt of high dielectric capacity. In this case, however, it appears that only a redistribution of silver atoms should take place, since for each electron emitted by a silver atom a silver cation remains. Delicate methods for determination of free silver have been developed by Weigert and Lühr which may give quantitative data on this point.

"The status of dye sensitizing is much the same. A great amount of valuable technical information has been obtained and the number and efficiency of such optical sensitizers enormously increased, but the theory of their action is still obscure. The hypothesis of collisional transfer of energy has been applied here also, but is difficult to test. Leszinski, working in Eggert's laboratory, found that the absorption of light by one dye molecule (of erythrosin) could give at least 20 silver atoms on visible decomposition of silver halide. This requires something more than the collisional transfer of energy quanta—namely, a superposed chain reaction—the mechanism of which is not clear. Sheppard and Crouch have found two conditions of adsorption of basic dyes to silver bromide. One consists in approach to monomolecular layer formation, provided the dye is in true solution—i.e., if its 'concentration' at equilibrium is below its actual solubility. Above this—

¹ Ind. Eng. Chem., **22**, 555 (1930).

from colloid solutions of the dye—multimolecular adsorption of the dye molecules occurs. The maximum effect in optical sensitizing is reached far below this level of concentration and much below formation of a complete molecular layer. This indicates that sensitizing is effected by monomolecular patches of relatively few dye molecules. If the collision hypothesis is correct, it would seem that any strongly adsorbed dye could act as a sensitizer for its own absorption band. This is not the case. Although many classes of dyes give more or less feeble sensitizing, many strongly adsorbed dyes do not sensitize. Actually only two groups of dyes are now practically employed as sensitizers, the phthalein and the polymethine or cyanine type dyes.

"The presence in these of co-ordinatively saturated and co-ordinatively unsaturated atoms of nitrogen (also oxygen, in phthaleins) joined by a system of conjugated double bonds, makes rather plausible Baur's¹ theory of photosensitizing as an intramolecular electrolysis. A continuous system of conjugated double bonds is equivalent, in a single molecular structure, to a metallic conductor, since it permits transmittance of an electron along it according to the potential at a given point. The length of this system will determine the mean resonance frequency for light waves. Given two atoms, which are capable of two valency stages, sufficiently separated by such a chain, then electrolytes could be attached by 'Anlagerung,' and activation by light absorption could bring about actual decomposition.

"My colleague, A. P. H. Trivelli, has discussed² a similar possibility for micellar photo-elements, with colloid silver and silver sulfide, which would bring optical sensitizing by these in line, formally at least, with Baur's interpretation. Historically, it appears that this is in direct line of descent through Bancroft with Grotthuss' electro-chemical theory of photochemical reactions. This is the sad part of the theory, that it is a return to a quite primitive conception."

Sheppard is quite right in saying that one possibility is a return to what he considers the primitive concepts of Grotthuss. As a matter of fact Grotthuss gave the true explanation of Vogel's discovery implicitly over a half century before the phenomenon was observed. Grotthuss found that ferric chloride in water is practically non-sensitive to light because the reduction of ferric chloride to ferrous chloride would be reversed at once by the free chlorine or by its reaction products with water. Grotthuss did not speak of decomposition voltages or depolarizers because they had not been invented at that time; but he dissolved ferric chloride in alcohol and exposed again to the light.³ "In alcoholic solution ferric chloride is reduced by light to ferrous chloride. The reason for the difference is that chlorine reacts readily with alcohol but not readily with water. On the other hand Grotthuss found that ferric sulphate in alcohol was only acted on very slowly by light. The reason for this is that alcohol is not a good depolarizer for oxygen but is a good one

¹ *Helv. Chim. Acta*, 1, 186 (1918).

² *Z. wiss. Phot.*, 26, 381 (1929).

³ Bancroft: *J. Phys. Chem.*, 12, 230 (1908).

for chlorine. . . . The light-sensitiveness of all substances is increased by the presence of a suitable depolarizer, and, in many cases, we get light-sensitiveness only in presence of depolarizers."

Grotthuss was using a colorless depolarizer in the case of alcohol and therefore the light absorbed by the alcohol had little or no effect. In his experiments the ferric chloride was oxidizing the alcohol. If he had used a colored depolarizer, the light which was absorbed by the depolariser would have activated it and would have increased its tendency to reduce ferric chloride. If this activation were sufficient, he would have got photochemical reduction of ferric chloride by the light which was absorbed by the colored depolarizer, in other words the Vogel phenomenon.

We have been familiar with this for years in another form. If we do not use ultra-violet light, which might cause formation of ozone, the light effective in the oxidation of dyes¹ by air is light which is absorbed by the dye. The dye reduces the oxygen instead of the oxygen oxidizing the dye. We do not ordinarily speak of this as a case of optical sensitization of oxygen by a dye because we are not interested in the reduction of oxygen by light, whereas we are interested in the reduction of silver bromide by light.

The general theory of the Vogel phenomenon was formulated clearly² over twenty years ago.

"The experiments of von Hübl³ show that the sensitizing action of a dye may be modified very much by the quantity of the dye in the gelatine acting as a screen. A photographic plate is only sensitized by a dye if the silver bromide is itself colored. . . . The Grotthuss theory requires that the sensitizers should be depolarizers. They must be decomposed by light and must either be reducing agents or must be converted into reducing agents by light. It is not necessary that the order of light-sensitiveness should be identical with that of the sensitizing power. This latter depends on the [chemical] potential while the rate of decomposition depends also on the unknown 'chemical resistance.' On the other hand, a general approximation between the light-sensitiveness and the sensitizing power is to be expected and is found. Dyes, which stain silver bromide and which are not depolarizers, directly or indirectly, are not sensitizers."

Putting this into more modern phraseology, an optical sensitizer for a silver bromide film is a colored substance which is adsorbed by silver bromide, which does not bleed appreciably into gelatine, and which is either a powerful enough reducing agent to produce a latent image with silver bromide under the influence of light, or is converted by light into a reducing agent powerful enough to produce a latent image with silver bromide. This of course does not cover cases where there is fluorescence.

This way of looking at things has not been popular with the mathematical photochemist, as is shown by Sheppard's remark already quoted. Neither

¹ Bredig and Pemsel: *Archiv wiss. Photographie*, 1, 33 (1899); Bancroft: *J. Phys. chem.*, 12, 257 (1908).

² Bancroft: *J. Phys. Chem.*, 12, 353, 375 (1908).

³ *Jahrbuch der Photographie*, 10, 289 (1896).

H. S. Taylor nor Sheppard look with favor on the concept of depolarizers, perhaps because nobody has yet applied the quantum theory to it. While one could attack this particular problem on the basis of the original Grotthuss formulation that the action of a ray of light is analogous to that of a voltaic cell, there are cases to which this generalization cannot be applied satisfactorily and the modified Grotthuss theory¹ now reads:

1. Only those rays of light which are adsorbed can produce chemical action.
2. Light which is absorbed by a substance tends to eliminate that substance. It is a question of the chemistry of the system whether any reaction takes place or what the reaction products are.

It therefore seemed desirable to do a few experiments in order to show again how simple the phenomenon really is. Contrary to the general view of modern scientific men, we believe that the easiest experiments are the best and that there is no point in doing a difficult experiment unnecessarily.

If we eliminate the gelatine, we avoid the danger of bleeding and the consequent formation of a color screen. If we eliminate the solid silver bromide, we avoid the question of adsorption. If we start with a system which is practically insensitive to light, we avoid differential development and we can expose as long as we like, thus making it possible to study the behavior of dyes which fade very slowly in the light. If we can use a qualitative test for showing reduction, we avoid the time necessary to do quantitative analyses.

This meant harking back to the experiments of Grotthuss. He had shown that there is no reduction by light of ferric chloride in aqueous solution and we have confirmed that, so far as exposures of six hours are concerned. On adding a dye in the absence of air and exposing until the dye was entirely decomposed, it then became a simple matter to test for ferrous chloride with potassium ferricyanide. In every case in which the dye faded, there was a reduction to ferrous salt, just as the theory required.

Since the mathematical photochemist will probably feel that ferric chloride in solution is too different from solid silver bromide in gelatine to justify reasoning from one case to the other, we decided to meet him half way, though without admitting the validity of his hypothetical contention. We therefore used solutions of silver nitrate in water, hoping that perhaps reasoning from a silver salt would seem less far-fetched to the constitutional objector, even though the silver salt was soluble and there was no gelatine.

Mellor² says that "silver nitrate blackens if exposed to light, but not if organic matter be rigorously excluded" (ady³ says that silver nitrate in the pure state is not altered by light).

We obtained the purest silver nitrate possible and exposed it for six hours in the carbon-arc Fade-ometer. At the end of this time the solution was still colorless and unchanged by light. When metallic silver was precipitated in the presence of a dye by the action of light, we knew, therefore, that this was due to the reducing action of the activated dye.

¹ Bancroft: J. Phys. Chem., **32**, 529 (1928)

² "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," **3**, 463 (1923)

³ "Inorganic Chemistry," 474 (1912).

All exposures were made in a Fade-ometer rather than in sunlight to insure uniformity and duplication of conditions. A Fade-ometer is a commercial instrument operating on 220 volts A. C., containing a glass-enclosed carbon arc and a means of holding the samples at a fixed distance (10 in.) from the source of light. This machine is made by the Atlas Electric Devices Company. Columbia violet carbons were used, and the globe enclosing the arc was of Corex glass, which transmits more ultraviolet light than any other type. The transmission percentage for the different wave lengths was worked out by the Bureau of Standards at Washington, the measurements being made on May 25, 1926 between eleven and twelve a. m. Their results are given in Table I.

TABLE I

Spectral Range	% of Total Radiation	
	Arc	Sun
170 to 320 $m\mu$	0.0	2.0
320 to 360 $m\mu$	2.0	2.8
360 to 480 $m\mu$	18.5	12.6
480 to 600 $m\mu$	9.3	21.9
600 to 1400 $m\mu$	16.5	38.9
1400 to 4200 $m\mu$	22.1	21.4
4200 to 12000 $m\mu$	31.6	.4

The arc stream developed produces a spectrum containing qualitative rays of natural June sunlight, but the light of the Fade-ometer is more intense than sunlight, twenty hours exposure in the instrument being equal to about 50 hours in June sunlight. The humidity was controlled by keeping three receptacles under the globe constantly filled with water. Because a great deal of heat was generated by the arc, two fans placed on opposite sides of the instrument were kept constantly running and two of the windows were left uncovered in front of them so that the air currents resulting would meet in the center and carry the heat out the top of the Fade-ometer. The temperature conditions were satisfactory under these conditions. (The newer model Fade-ometers come all equipped with suction fans for heat removal.)

We wanted to expose our solutions in the absence of oxygen so that if a reaction did occur we could be sure that it was due to the sensitizing power of the dye and not merely to oxidation. It was therefore necessary to remove all the air from the flask, which we did by displacing it with nitrogen. In order to do this we needed an exposure flask having an inlet tube with a stopcock and an outlet stop-cock. We used 50 cc absorption flasks which had had stop-cocks sealed onto the side arm leading in. These flasks come with ground glass stoppers which can also be used as stop-cocks. It was therefore possible to bubble the nitrogen in through the side arm (which extends to the bottom of the flask) up through the solution, and out through the stopper, thus effecting removal of the air. The flasks used for the exposure of the ferric chloride

solutions were made of German soft glass, but those used for silver nitrate solutions were of pyrex, which has a higher transmission power for ultra-violet light.

The nitrogen gas used was prepared from liquid air (Matheson Co.) and might therefore contain traces of oxygen. For this reason the nitrogen was passed through two absorption bottles each containing 150 cc alkaline pyrogallol. Before discontinuing the treatment with the nitrogen gas, a small flask containing copper gauze in a solution of cuprous tetrammino sulphate was attached to the end of the system. If the evolving gas still contained traces of oxygen this solution would become blue.

Preparation of Pyrogallol and Cuprous Tetrammino Sulphate—

- 1.—800 gr. KOH in 1000 cc water solution.
150 gr. Pyrogallic acid per liter of KOH solution.
- 2.—Approximately 10% saturated copper sulphate solution
35% ammonium hydroxide (15N)
55% water

Put the mixture in a bottle with some freshly cleaned copper gauze, stopper, and shake until it becomes colorless. If the blue color does not disappear in half to three quarters of an hour, adjust concentration slightly by adding small amounts of ammonium hydroxide or water until it is such that the copper gauze can effect reduction to the colorless solution on further shaking in the stoppered bottle.

Preparation of Solutions—

All the dye solutions used in this research were of the same strength—.1 gr. per liter.

The ferric chloride solutions were 5% (by weight) ferric chloride and 95% distilled water.

The silver nitrate solutions were 5% (by weight) silver nitrate and 95% distilled water.

Procedure and Data for Ferric Chloride Solutions—

Five cc dye solution were added to forty-five cc ferric chloride solution, making a total of fifty cc, 10% of which is the dye solution of strength 0.1 g per liter. The actual concentration of the dye in the flask ready for exposure is therefore 0.01 g per liter, a very weak solution, which is desirable in sensitizing work as mentioned previously.

The air was then removed from the flask as described above, and the preparation placed in the Fade-ometer for exposure. After exposure the solution was tested for the presence of ferrous ions with potassium ferricyanide. The results obtained in these experiments are given in Table II.

In every case the dye caused a sensitizing of the ferric chloride solution which resulted in its reduction to ferrous chloride.

Water solutions of the same dyes were exposed simultaneously with the ferric chloride solutions to see whether the observed effects were partially due to the natural fading of the dyes or solely to the reaction with ferric chloride. It was found that the colors of the dyes in water did not change noticeably in

the time of exposure, and we conclude that the action was entirely due to the sensitization of the FeCl_3 by the dyes. It was found later, however, that all of the dyes were sensitive to light on long enough exposure. This is in accordance with Vogel's theory which holds that all depolarizers must be light-sensitive.

TABLE II
Ferric Chloride

Name of Dye	Time of Exp.	Color Change	Test for Fe^{++} ion
Eosin	5 hrs.	Sol'n became cloudy.	Positive
Methyl Violet	4 hrs.	Green to orange of FeCl_2 sol'n in 45 minutes.	Positive
Magenta		Changed immediately on addition of dye—5 minutes.	Positive
Methylene Blue	4 hrs.	Yellowish green to greenish amber in 45 minutes.	Positive
Acid Green	40 min.	Dull olive green to orange.	Positive
Cardinal Red	1 hr.	Dark orange brown to orange.	Positive
Brilliant Green	1 hr.	Light olive green to orange.	Positive
Aurine	2 hrs.	Dye too weak to change color of FeCl_3 in first place.	Positive
Sensitizers from Eastman Kodak Company			
Erythrosin	30 min.	Reddish orange to yellowish orange.	Positive
Piancyanol	30 min.	Cloudy orange to clear reddish orange.	Positive
Orthochrome T Bromide	45 min.	Cloudy orange to clear reddish orange.	Positive

These experiments on ferric chloride solutions and dyes illustrate that sensitizers are reducing agents in the broad sense of the term, and that the light-sensitiveness and sensitizing power of a dye need not run strictly parallel; also that if the limiting physical conditions connected with a photographic plate be lessened, the number of dyes that can act as sensitizers will be increased. All the results are in accordance with the principles laid down by Grotthuss, and uphold Vogel's ideas.

Before proceeding with the repetition of the above tests on silver nitrate solutions, we performed a few experiments with color screens to bring out the fact that it is the light absorbed by the dye which actually is effective in bringing about the sensitizing and the chemical reaction. To test this, samples of ferric chloride and dye, with and without a color screen of the same dye were exposed. According to theory, if methyl violet is used as a sensitizer, it is the light absorbed by the methyl violet that would cause the sensitizing action. Therefore a small test tube containing ferric chloride and methyl violet (in the same proportions as in previous experiments) was tightly corked and submerged in a flask containing methyl violet in pure water, the concentration of the dye in the outer flask being greater than that used as sensitizer.

The combination was then exposed simultaneously with a flask containing ferric chloride and sensitizer without the color screen. The time required for fading was compared, and as expected, was greater when the color screen was present to absorb part of the rays normally absorbed by the sensitizer. This experiment was repeated on several different dyes, always with the same result. The inner solutions were 10% dye solution and 90% ferric chloride solution, while the color screens were 15% dye solution and 85% water. The tabular results are given in Table III.

In every case it took longer, and in 3 out of 5 cases at least twice as long, for the dye to act when the color screen was used as when exposed directly. This shows conclusively that it is the light absorbed by the sensitizing dye which is effective, for if it be withheld, the action is greatly retarded.

TABLE III
Ferric Chloride

Name of Dye	Fading Time with Color Screen	Fading Time with- out Color Screen
Methyl Violet	75 min.	20 min.
Methylene Blue	230 min.	185 min.
Acid Green	20 min.	10 min.
Cardinal Red	30 min.	20 min.
Brilliant Green	30 min.	10 min.

However, if the action were entirely due to the light absorbed by the dye, there should have been no action at all in the presence of the color screen. We concluded, therefore, that the light absorbed by the ferric chloride must also be effective. The experiments were repeated, using two color screens, one of dye as before, and an additional one of ferric chloride solution. The dye solution was placed in a large test tube and a smaller test tube containing ferric chloride immersed in it. A still smaller tube containing the dye-ferric chloride solution was then dropped into the center and we had a system consisting of a dye color screen, a ferric chloride color screen, and a solution of dye in ferric chloride. The relative sizes of the tubes selected were such that there was about a half centimeter between their walls, thus allowing a sufficiently thick color screen.

The concentrations were the same as those in the previous experiments with color screens. The ferric chloride used as a screen was a 7½% solution.

TABLE IV
Ferric Chloride

Name of Dye	Test for Ferrous Ions	
	Direct Exp.	With Color Screens
Methyl Violet	+ in 20 min.	— in 40 min.
Methylene Blue	+ in 60 min.	— in 120 min.
Acid Green	+ in 10 min.	— in 20 min.
Cardinal Red	+ in 20 min.	— in 40 min.
Brilliant Green	+ in 10 min.	— in 20 min.

The results obtained with double color screens were very satisfactory and are given in Table IV.

Under these conditions there is absolutely no action, even with twice as long an exposure. This shows conclusively that the action is due partially to light absorbed by the dye and partially to light absorbed by the ferric chloride, and that *only light which is absorbed* is effective in producing the photochemical action.

Procedure and Data for Silver Nitrate Solutions—

The next step was to apply our principles to silver nitrate solutions. Since the restrictions concerned with silver bromide plates are not involved, we should expect any light-sensitive dye which was or became a sufficiently powerful reducing agent to sensitize silver nitrate as well as ferric chloride. As it was out of the question to test every light-sensitive dye, we tried to choose a group which would be representative of all the dyes. We have several basic dyes, several acid ones, and at least one from every important group in dye classifications. Eosin and erythrosin gave precipitates with silver nitrate and therefore were not tested. Pinacyanol and orthochrome T bromide are sufficiently strong reducing agents to reduce a silver nitrate solution in the dark.

The only difference between the performance of these experiments and those on ferric chloride is that 5% silver nitrate is substituted for 5% ferric chloride, and 75 cc pyrex flasks were used in place of the 50 cc soft glass ones. The total volume used was therefore increased to 75 cc but the proportions were unaltered. Oxygen was removed as before. The results obtained are given in Table V.

TABLE V
Silver Nitrate

Name of Dye	Time of Exp.	Color Change	Silver Deposited
Magenta	5 hrs.	Red to colorless	Yes
Methyl Violet	5½ hrs.	Purple to colorless	Yes
Brilliant Green	5½ hrs.	Green to colorless	Yes
Methylene Blue	45 hrs.	Blue to very pale blue	Yes
Alkali Blue	5½ hrs.	Blue to colorless	Yes
Acid Green	5½ hrs.	Green to colorless	Yes
Cardinal Red	5½ hrs.	Pink to colorless	Yes
Eosin	None	Colored precipitate separated out. Colorless supernatant liquid	
Erythrosin	None	Cerise colored precipitate settled out immediately. Supernatant liquid colorless and clear	
Pinacyanol	None	Purple to colorless in 3 or 4 minutes —before air could be removed	
Orthochrome T Bromide	None	Scarlet to colorless in 3 or 4 minutes —before air could be removed	

To make sure that the black grains deposited were really metallic silver, the colorless solution containing them was stirred up and a large drop of it placed on a ground glass plate and allowed to evaporate. A dark spot resulted which, when slightly burnished, gave a silvery, metallic lustre. This verified the fact that the precipitate was silver and not some oxide or impurity. Eosin and erythrosin give a precipitate with silver nitrate, and therefore cannot be used in these tests.

Every dye exposed with silver nitrate was found to be a sensitizer as we expected and the results on the silver nitrate solutions are just as satisfactory as those obtained with ferric chloride.

Since ammoniacal copper oxide solution can be reduced to a colorless cuprous oxide solution, we thought it should be possible, by varying the alkalinity and nature of the reducing agent, to get a solution which would not decolorize in the dark, but would in the light. By cutting down a little more we might get a solution which would not decolorize in the light, but which would lose its blue color on addition of a light-sensitive color and exposure to light. This was therefore tested out and found to be possible.

A solution containing 20 cc 5% CuSO_4 , 10 cc 3N NH_4OH , and 70 cc water was prepared. Ten cc portions of this solution were titrated with a $\frac{1}{2}\%$ solution of phenyl hydrazine. It was found that when 2 cc were added to a ten cc portion of the ammoniacal copper oxide solution it would fade in the light but not in the dark (within twenty minutes).

The amount of phenyl hydrazine was then cut down to 1.8 cc and the blue color did not disappear upon a twenty-minute exposure in the Fade-ometer. The experiment was then repeated, and a flask containing one cc of eosin was also exposed simultaneously. At the end of twenty minutes neither flask was colorless, but the one containing the eosin was much more faded than the one without it.

This work was repeated using methylene blue and methyl violet instead of eosin. The results were the same. The flask containing the dye always faded faster. If left in the light long enough, they would both become colorless, but the one containing the sensitizer would always lose its blue color more rapidly.

Throughout the entire research we have obtained results which fall in line with the fundamental theories and overthrow Carey Lea's disproof of Vogel's law. However, there is no doubt that his work was carefully done and that he actually did obtain the results stated, but there are several considerations which may account for them.

Some of the observed facts that led him to conclude that the sensitizing action of a dye bears no relation to its color were:

- 1) Coralline, a red dye, increased the sensitivity of silver chloride and silver bromide for red rays.
- 2) Coralline, a red dye and therefore expected to absorb green and blue rays, gave no increase of sensitivity to either green or blue light when used with silver iodide.

3) With silver bromide, the substances giving the greatest increase in sensitivity for the more refrangible half of the spectrum were colorless.

4) Some dyes, among them coralline, decreased sensitivity to some rays for which they were expected to increase it.

The first objection may be accounted for by the fact that coralline is not a pure substance but a mixture of two dyes and of their oxidation products. Consequently the age as well as the method of preparation cause variations in the composition. Therefore, although a spectrum analysis of our coralline did not show any absorption bands in the red, it is still possible, and probable, that Carey Lea's coralline did absorb in the red. This would naturally account for its causing an increase in the sensitivity toward red rays.

The fact that the coralline showed no increase in sensitivity of silver chloride toward green and blue light is nothing to be alarmed about, because it has always been recognized¹ that, not even with light-sensitive substances, are all of the absorbed rays necessarily active. The presence of absorption bands in the green and blue by no means means that a substance will be sensitive to green and blue light, but only that it *may* be. It is still true that whatever light does activate it is light which is absorbed, and the action is therefore still related to the color, since the light absorbed indirectly determines the color.

It is perfectly possible that the colorless substances which caused increased sensitivity were reducing agents even in the dark. They did not give specific action for specific colors.

The decrease in sensitivity observed in some cases can also be explained by the fact that not all the rays absorbed are necessarily active in producing a chemical effect. They may be absorbed, may not act themselves, and may serve as a color screen for rays of the active absorption band. Let us imagine a dye having absorption bands in the red and in the green which is only sensitive to green light. The red light absorbed is inactive, and yet it may serve as a color screen for the green light which would be active if it could get in. Such a dye might easily cause a decrease in the sensitivity of silver chloride for green light.

Carey Lea should have concluded, not that the color of a dye bears no relation to its sensitizing power, but that the color, or more especially, the light absorbed, is the important and determining factor, although we cannot, as yet, predict what fraction of the light absorbed will cause activation.

The general conclusions of this paper are as follows:—

1. The Grotthuss theory enables us to account for Vogel's results.
2. An optical sensitizer in photography—in cases in which fluorescence is barred—is a colored substance which is adsorbed by silver bromide, which does not bleed into gelatine sufficiently to form a color screen, and which is either a powerful enough reducing agent to produce a latent image with silver bromide when activated by light or is converted by light into a reducing agent sufficiently powerful to produce a latent image with light.

¹ Bancroft: J. Phys. Chem., 12, 209 (1908)

3. In the cases so far studied it is probable that the silver bromide is reduced by the activated dye and not by a reaction product.

4. In order to simplify the problem we have eliminated the gelatine, the solid silver bromide, and the sensitivity to light in the absence of dye. Aqueous solutions of ferric chloride and of silver nitrate meet these requirements.

5. It has been found possible to do optical sensitization of ferric chloride and of silver nitrate with a number of light-sensitive dyes.

6. It is possible to adjust the concentrations of a solution of ammoniacal cupric oxide and phenyl hydrazine, so that the solution is stable in the dark and bleaches in the light. Addition of sodium eosinate produces optical sensitization.

7. It has been known for years that dyes would act as optical sensitizers for oxygen; but we have not called them that because most people have been considering the oxidation of dyes and not the reduction of oxygen.

8. Carey Lea's criticisms of Vogel's theory are based chiefly on misconceptions, but probably in part on the use of unknown dye mixtures.

Cornell University.

THE ADSORPTION OF ORGANIC MATERIALS TO THE SILVER HALIDES*

BY S. E. SHEPPARD, R. H. LAMBERT AND R. L. KEENAN

Introduction

Adsorption at liquid-solid interfaces has been studied¹ for many years. The mechanism is fundamentally more complex than at gas-solid interfaces because of adsorbent, solution, and adsorbed material of the system. Still other factors enter when a crystalline adsorbent is considered as compared with amorphous material.

The effects of adsorption in the preparation of photographic silver halide emulsions are in evidence at many stages of the process. The size and distribution of grains is affected by common ion action² and by concentration of gelatin.³ The shape of grains can be altered enormously by foreign materials such as urea, dyes, *etc.*⁴

In the photographic emulsion adsorption of gelatin has a protective effect with respect to reduction of the silver halide apart from its effect on size-distribution, and shape. Reinders and Nieuwenburg⁵ found that reduction of silver chloride by ferrous citrate was distinctly restrained by a gelatin concentration as low as 0.0012 per cent of the total system. Sheppard suggests⁴ that specific orientation of active groups to silver halide surfaces may account for this protective colloid property.

A further adsorption effect is the general sensitizing imparted by gelatin to the silver halide grain. Gelatin contains certain organic sulfur-containing bodies which, on coming in contact with silver halide surfaces, react to form complex compounds which are more or less unstable. These decompose to form silver sulfide nuclei which in turn act as sensitivity centers for developing the latent image produced by light.⁶ This sensitizing, so fundamental for photographic emulsion preparation, is necessarily controlled by adsorptive forces acting at the solid-liquid interface.

An integral part of photographic emulsion is optical or color sensitizing by adsorption of dyes. A considerable study has been made of the mechanism of dye deposition on crystalline material.⁷ Adsorption of dyes depends on the basic or acidic nature of the dye and on the polarity of the surface of adsorbent.⁸ The nature of the dispersity of dyes and the effect of concentration in solution on dispersity are still little known in most cases.

Finally, adsorption plays a primary role in the formation and development of the latent image.⁹ Adsorption at silver halide surfaces must undoubtedly have an enormous influence on the free energy available for crystal growth, as described by Kossel.¹⁰

* Communication No. 481 from the Kodak Research Laboratories.

Adsorption of Inorganic Ions to the Silver Halides

A summary of previous work on the adsorption of materials by the silver halides is given by Wulff and Seidl.¹¹ The study made by Fajans¹² and co-workers in which inorganic ions were adsorbed gives evidence that photochemical decomposition is greatly influenced by common ion adsorption. Fajans' observation that every fourth to tenth atom of bromine in the silver bromide lattice has adsorbed a silver ion from a silver nitrate solution cannot be regarded as the same for other silver salts in solution since Beekley and Taylor¹³ find that adsorption of silver ion is dependent on the anion and roughly, that the greater the solubility of silver salt the less adsorption results. Extraneous electrolytes, furthermore, have a pronounced effect on the adsorption process.

Fajans¹⁴ describes adsorption as a dehydration of the deposited ion, thus causing a definite energy change in the silver halide lattice. He also obtained evidence of anion deformation by non-noble metal cations, thus giving polarization to the surface layer of the crystalline adsorbent.

Others have studied adsorption of inorganic salts to the silver halides, among whom are Luther¹⁵ (cuprous ions on silver bromide), Lottermoser (common ion), and Paneth, (radium on silver chloride). The latter found no adsorption in accordance with his rule. Fajans and co-workers found adsorption of thorium B to silver halide sols stabilized by halide ions but none when stabilized by silver ions. Adsorption of thallium ions was studied by Wulff and Seidl.¹¹

Adsorption of Dyes to the Silver Halides

Only a few experiments have been made quantitatively on the adsorption of dyes to the silver halides and yet this question is of paramount importance for optical and chemical sensitizing or desensitizing. Probably the first systematic study was that by Kieser.⁷ Some of his conclusions will be discussed later. Others in this field were Fajans⁸ and co-workers, Lüppö-Cramer,¹⁶ Wulff and Seidl,¹¹ and Sheppard and Crouch.¹⁷

Gelatin Adsorption to the Silver Halides

Eder¹⁸ was the first to observe definite retention of gelatin by silver halides. He reported 0.5 per cent gelatin was still retained after repeated centrifugations and washings with hot water. Reinders¹⁴ reported 0.1 per cent adsorbed where ammoniacal silver chloride was the adsorbent. No other data could be gleaned from the literature.

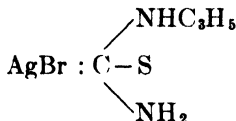
Adsorption of Substances which form Compounds with the Silver Halides

Little seems known of an adsorption phase in the case of solutes forming definite addition compounds with the adsorbent (solid).

In the silver halides, one can refer to the researches of Kieser on adsorption of dyes which form slightly soluble compounds with the cation of the adsorbent. Kieser recognizes a form of adsorption in which the law of mass action is

supposed to hold. For example, silver iodide with tetra-iodo-fluorescein gives very little intensity, while silver chloride adsorbs so strongly that quantitative results can be easily obtained. With sodium fluoresceinate and silver halides, the solubility of the silver fluoresceinate was so high that no adsorption could be measured.

We have made some experiments in the case of allyl-thiourea and silver bromide. These form a double compound (1:1)



of low solubility (2.3×10^{-4} gm. mols. per liter at 25°C ., cf. Sheppard and Hudson²⁰) and probably also 1:2 and 1:3 compounds of much higher solubility, since high concentrations of allyl-thiourea "fix" or dissolve silver bromide. We were interested in seeing whether up to 1:1 equivalent of allyl-thiourea would be completely taken up by solid silver bromide. This did not appear to be the case, at least, in a reasonable time. Instead, considerably below molecular equivalence, the silver bromide grains became agglutinated, a plastic mass forming. Apparently before the whole grain of silver bromide is converted to the 1:1 compound, the outer layer of this is carried up to the higher order compounds, and some is dissolved, since appreciable silver was found in the solution.

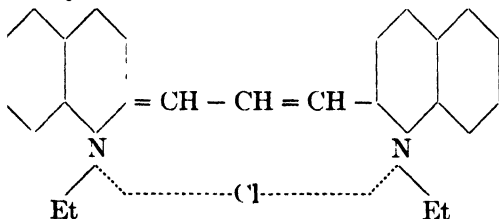
Experimental Results

The experiments to be recorded are preliminary to a more extensive investigation of adsorption as related to the photographic process. In this, the adsorption of sensitizing dyes is of great importance.

An example of one such experiment is shown in Fig. 1. Pinacyanol and orthochrome T are compared under somewhat similar conditions.

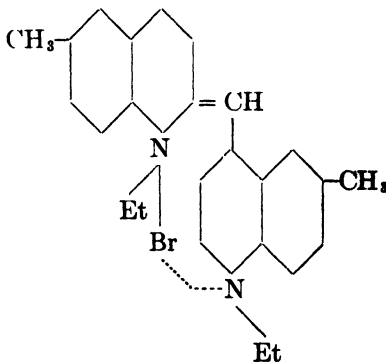
The formulas and molecular weight are:

Pinacyanol



Mol. wt. 388

Orthochrome T



Mol. wt. 436

They are both basic dyes in which the dye molecules serve as cation, and halogen atoms as anion.

Sheppard and Crouch found that simple extraction with chloroform gave good quantitative data for orthochrome T dye adsorption to grains. A check was possible since final dye concentration could be obtained by centrifuging the grains and removing a portion of the solution for dye estimation.

In the case of pinacyanol an added difficulty arose since the coefficient of distribution between chloroform and water is 0.20 at pH 6.0 to 7.0, while for orthochrome T, 5.0 was found by Sheppard and Crouch at about the same pH. We found the ratio for pinacyanol could be raised by salting out with sodium chloride, the best result being obtained at 25 gm. sodium chloride per 100 c.c. dye solution. At this salt concentration 85 per cent of dye passed into the chloroform layer whose volume was fixed at 25 c.c. This partition was independent of dye concentration within the limits studied.

Furthermore, since sodium thiosulfate was used to dissolve the grains after separation from the dye solution, a study was made of the ratio of sodium chloride and sodium thiosulfate in the dye solution. It was found that if 10 gm. of sodium chloride were replaced by an equal amount of sodium thiosulfate, the same distribution coefficient was obtained as if only sodium chloride was present.

The silver bromide grains were prepared in an amount sufficient for the whole series of experiments. They were precipitated in solution of gelatin in an excess of potassium bromide present throughout. After two washings on the centrifuge, a definite amount of gelatin still adhered to the grains, of which more will be said later. This amount is equivalent to 4 mg. gelatin per gram silver bromide. A sample was taken for photomicrographic grain size determination.

Samples of silver bromide (0.34 gm.) were taken up in 100 c.c. of dye solution. The whole was well shaken mechanically for several hours at 25° C., after which the grains were separated by centrifuging. As much as possible of the dye solution was removed without disturbing the deposited grains, and its volume was accurately determined. Its concentration was determined colorimetrically.

The grains were then treated with sodium thiosulfate and sodium chloride, as described above, and the amount of dye again determined colorimetrically. Accurate results were found only for dye determination on grains since the concentration in solution was invariably low. The values obtained were erratic owing to surface adsorption to the walls of the container. Much of this error could be eliminated by coating the interior of the bottles with a rather thick layer of paraffin.

Sheppard and Crouch¹⁷ calculated that one dye molecule was held for 2.3 bromide ions of the surface. This calculation necessarily assumes a certain state of the surface of the grains. Further, it assumed only projective area ($\times 2$) for the adsorbing area. From photomicrographs and certain assumptions, we now consider that *three* figures can be obtained for the ratio of dye molecule adsorbed to bromide ion: (1) One may assume adsorption to the octahedral face only, that is, to the projective surface and its opposite surface; (2) total surface may be obtained if the thickness of grains is known

and a definite shape is assumed as for example a thin disk; (3) if the edges are assumed to be cubic faces, then only half the silver bromide ions on the surface could be bromine ions and a third figure would be obtained. Calculating in this way one gets respectively the numbers 1.69, 2.78, and 2.24 for bromide ions per one dye molecule adsorbed in the case of pinacyanol (Fig. 1).

The value 1.69 for octahedral adsorption is still far from unity, that is, monomolecular adsorption. This might, however, be accounted for by assuming that not all projective area is that of octahedral faces. This result may be compared (Table I) to the collected table of data taken from the paper by Wulff and Seidl.

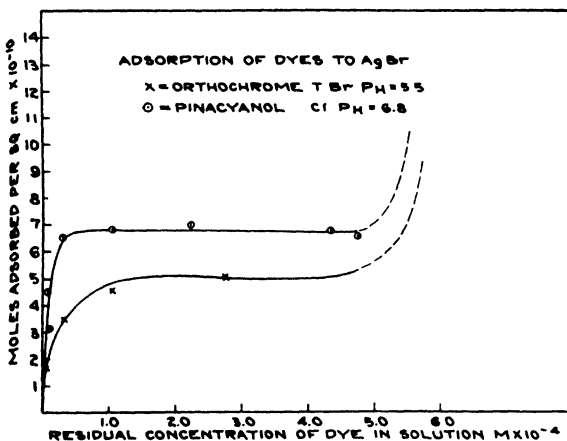


Fig. 1

It is observed that less than one molecule of material is adsorbed per ion of adsorbent. In the case of a large dye molecule such as those of the carbocyanines or isocyanines it is conceivable that there is scarcely room for an ion-to-ion union since the cross section of the quinoline group of the dye is itself as large or larger than the interionic distances of the silver bromide lattice, that is, about 6 Å, and there are two such quinoline groups per molecule of dye. Silver ion adsorption, however, could conceivably be equal to that of bromide ion on the silver bromide crystal surface. Fajans did not find this to be the case and Beekley and Taylor find that silver ion adsorption varies for various soluble silver salts.

That adsorption is dependent on electrolyte concentration and especially common ion concentration has been pointed out many times. Fig. 2 gives such an instance as observed by Wulff and Seidl¹¹ for the adsorption of *resorcin* to silver bromide. Silver ion increases the adsorption of this and bromine ion decreases the adsorption. Cases are shown where certain electrolytes have no effect on dye adsorption. The salts indicating no effect on adsorption in Fig. 2 are phosphates, borates and carbonates.

The effect of hydrogen ion concentration depends on the acidic or basic nature of the dye. In Table II are shown qualitative results of adsorption for

TABLE I*

Vergleich der Besetzungsdichten bei Ionenadsorption und Farbstoffionenadsorption

Adsorbens	Adsorbat	Zahl der Ionen des Adsorbates Zahl der adsorbierenden Ionen des Adsorbens	Autor und Bemerkungen
AgBr- Präparat III	Methylenblau in neutraler Lösung bei Sättigung der Oberfläche	1:8	Diese Arbeit
"	Tl ⁺ in n/10-NaOH bei Sättigung der Oberfläche	1:3	
"	Resorcinat in n/10-NaOH		
	0.05 M/L = 0.55%	1:1.7	
	0.025 " = 0.28%	1:2.5	
	0.01 " = 0.11%	1:6	Interpoliert aus der Adsorp- tions-Kurve 10 d. Fig. 3
PbSO ₄	Ponceau 2 R bei Sättigung	1:1.1	Ungerechnete Werte nach F. Paneth ³⁵ Radio-Elements as Indicators 1928, S. 71
PbS	"	1:5.0	
"	Methylenbl. B ex- tra bei Sättigung	1:5.5	
"	Methylblau HB bei Sättigung	1:3.5	
AgBr	Orothochrom T	1:2.3	S. E. Sheppard u. H. Crouch ³⁴
"	Erythrosin	1:3	O. J. Walker ⁵
"	Ag ⁺	1:6	K. Fajans u. W. Frankenburger ¹

* From Wulff and Seidl: Z. wiss. Phot., 28, 239 (1930).

pinacyanol, a basic dye, and for dichlorofluorescein, an acid dye, with excess common ion in alkaline and acid solution.

It is rather remarkable that pinacyanol, a basic dye, tends to adsorb in an acid medium and in the presence of Ag^+ . If, however, a study of silver ion complex is made, Fig. 3, it will be observed that such complexes do occur with the basic dye as well as with an acid dye such as erythrosin.²¹

The method is essentially an electrometric estimation of Ag in the presence of dye by means of a concentration cell. The deviation from zero denotes the amount of complex ion formation.* The curves for the two dyes are not comparable since the concentrations are not the same.†

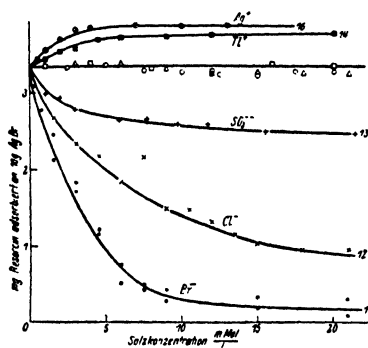


FIG. 2

From Wulff and Seidl: *Z. wiss. Phot.*, **28**, 239, (1930).

TABLE II

Influence of pH and Common Ion Excess on Dye Adsorption

	pH	Excess Br^-	Excess Ag^+	
Pinacyanol (Basic Dye)	5.0	+	+	+
	7.5	+	+	-
Dichlorofluorescein (Acid dye)	5.0	-	+	+
	7.5	-	+	+

Since silver ion complexes do form with some basic dyes it can be seen that adsorption is possible for dye on silver halide in the presence of excess Ag^+ .

* The technique and data for this are to be published by Dr. W. Vanselow and one of us later.

† There is, however, another factor to be kept in mind. The pinacyanol studied was a chloride derivative, and accordingly, silver ion might combine chemically with the chloride of the dye. The result would depend on the solubility of AgCl as compared with silver dye compound. The solubility product of AgCl is taken as 1.56×10^{-10} at 25°C . Then, knowing the concentration of chloride ion which is assumed to be equal to the dye concentration for the total ionization of dye chloride, one can calculate the Ag^+ concentration. For dye concentration of $1:40,000$ this is found to be 2.5×10^{-6} . The observed Ag^+ concentration was approximately 3.0×10^{-7} , i. e., the observed Ag^+ concentration is about 0.1 that of the amount calculated.

This could be accounted for if as much as 1% of the dye halide was found to be bromide. Qualitatively, no trace of bromide was observed in the dye.

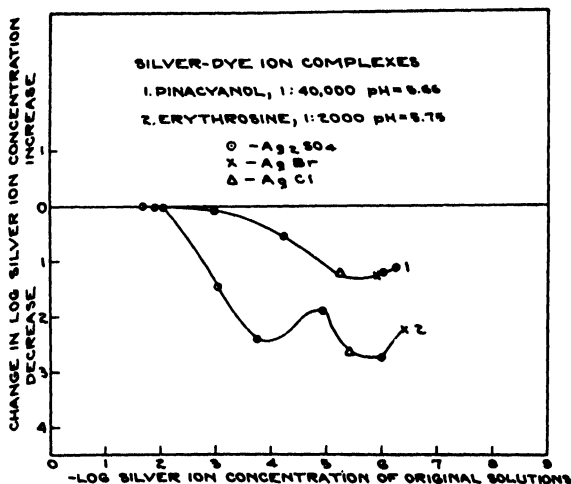


FIG. 3

Adsorption of Gelatin to Silver Bromide

In the first experiment a sample of silver bromide of known grain size distribution was prepared in gelatin solution by adding silver nitrate to potassium bromide in the presence of excess of the latter. A short after-ripening was given and the grains were removed from the solution by centrifuging.

The grains were taken up by distilled water at 50°C. and recentrifuged. This was repeated four times, but before each repetition a sample was removed for nitrogen determination. A repeat was run as a check. The nitrogen was determined, as described by Parnas and Wagner,²² by a micro-Kjeldahl method, the conversion factor of 5.6 being used for obtaining gelatin. The conclusion drawn is that after two washings no more nitrogen and therefore presumably gelatin can be removed (Table III).

TABLE III

Effect of Washing on Gelatin adsorbed to Silver Bromide Grains

Times Washed	pH Washing	mgms. Nitrogen per gm. Silver Bromide	mgms. Gelatin per gm. Silver Bromide
1	6.5	—	—
2	6.5	0.60	3.38
3	6.5	.61	3.40
4	6.5	.59	3.36

The effect of pH was next tried. In this case two washings were made with four different buffer mixtures. Table IV indicates slight decrease in adsorption only in acid medium. The value is, however, almost within the experimental error. pH then does not affect this phenomenon.

TABLE IV

Effect of pH on Gelatin adsorbed to Silver Bromide Grains

Sample	pH of Buffer	mgms. Nitrogen per gm. Silver Bromide	mgms. Gelatin per gm. Silver Bromide
1	4.0	0.57	3.19
2	6.0	.691	3.87
3	8.0	.632	3.54
4	10.0	.686	3.85



FIG. 4

Silver Bromide Grains from Gelatin Solution refluxed 6 hours

Since the areas of these grains have been determined by the photomicrographic method it is possible to calculate the thickness of gelatin layer assuming adsorption to the grain surface. Using the data obtained by two of us²³ for gelatin films on mercury the thickness found on these grains would correspond to a double layer of gelatin molecules.

On the other hand, it was later shown that about one-half of this gelatin could be removed by digesting with boiling water. This might imply that a monomolecular layer was actually present and that the rest of the gelatin was occluded in the grain; or, it is possible that a secondary layer is attached to the primary layer by weaker forces.

An experiment was next tried to determine whether all the gelatin could be removed, provided drastic means were taken. One sample of grains after the two washings was subjected to distilled water at 100°C., and another to 10 per cent sulfuric acid at its boiling point. The two were digested six hours. A motor-driven stirrer kept the grains in suspension during the operation. Photomicrographs (Fig. 4) of the resulting sample show little effect on shape or dispersion of the grains treated in distilled water, although profound changes occurred in the highly acidified solution.

On determining the nitrogen content only about one-half the gelatin retained on centrifuging and washing was present in the first case and less than one-tenth in the second. Further experiments are planned on the conditions of removal of gelatin from silver halide. The fact is clear in any case that some nitrogeneous material is tenaciously held to the grain (Table V).

TABLE V
Removal of Gelatin from Silver Bromide by hydrolyzing
with (a) Water and (b) Sulfuric Acid

Solution	1st Trial		2nd Trial	
	mgms. Nitrogen per gm. Silver Bromide	mgms. Gelatin per gm. Silver Bromide	mgms. Nitrogen per gm. Silver Bromide	mgms. Gelatin per gm. Silver Bromide
Distilled Water	0.22	1.23	0.273	1.53
Sulfuric Acid	0.061	0.34	.056	0.31

Finally, the effect of heat treatment of grains in 5 per cent gelatin solution has within limits shown no influence on adsorption. That is, raising the temperature from 40°C. to 60°C. and holding at either temperature for several hours does not affect the amount of adsorption.

A further experiment was now tried of adding silver bromide prepared without gelatin to various concentrations of gelatin solution. The silver bromide was prepared by adding silver nitrate to potassium bromide solution in the same manner for each sample. In Table VI the fourth sample was contaminated with 4 per cent nitric acid immediately after precipitation.

TABLE VI
Adsorption of Gelatin to 5 gm. AgBr
precipitated without Gelatin

Sample	Mg. Gelatin on Grains	Mg. Gelatin in soln.	Sample	Mg. Gelatin on Grains	Mg. Gelatin in soln.
1	4.0	11.0	4	28.9	104.0
	4.3	10.7		32.0	101.0
2	13.0	18.0	5	22.2	179.0
	14.2	16.5		23.9	178.0
3	14.0	53.0			
	15.9	52.0			

The sample, however, was washed with distilled water exactly as for the other samples. The higher adsorption value is presumably due to a higher total surface of grains, since little time was available for grain ripening as compared with that of the other samples. Checks were run at each gelatin concentration.

Summary

(1) The adsorption of pinacyanol, a basic dye, to silver bromide has been studied at pH = 6.8. Calculation of dye adsorbed to Br⁻ of the octahedral surface shows that 1.69 Br⁻ is necessary for one dye molecule. Calculations

are also made of total surface assuming both cubic and octahedral faces of silver bromide.

(2) Qualitatively it is shown that an acid dye such as dichlorofluorescein adsorbs to AgBr only for excess Ag^+ and mainly in an acid medium. Pincyanol adsorbs mainly in alkaline medium and for an excess of Br^- .

(3) Although allyl-thiourea does not follow any adsorption formula in its addition to the silver halides, the nature of the substances formed and the solubility of the various compounds is such that in some cases the reaction may not go on to completion.

(4) Gelatin is found to adsorb to silver bromide and when the halide is prepared in the presence of gelatin a layer of gelatin about the grain is so formed that it cannot be removed by boiling with water for many hours. Ten per cent sulfuric acid does not remove all the adsorbed material even after six hours' digestion, although the grains are markedly altered. Enough gelatin is still present after water digestion to amount to a monomolecular layer about the grain.

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THE PRECIPITATION OF PROTEINS IN PACKING HOUSE WASTES BY SUPER-CHLORINATION

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With the present increasing agitation against stream pollution, civic organizations, municipalities, and industries are more than ever faced with the problem of satisfactorily treating industrial wastes. Unfortunately, the biological methods that have been so successful for domestic sewage are not always applicable. The high strength of these wastes frequently makes the cost of these methods entirely excessive, and the chemicals present often interfere with biological activity. Even when it is possible to mix the two the results are frequently unsatisfactory. In facing this problem, sanitary engineers are therefore being forced to reconsider the almost discarded chemical precipitation method of sewage treatment.

Geo. A. Hormel & Co., packers at Austin, Minnesota, early arrived at the decision that biological methods were out of the question, and instituted a program of research to find the method of chemical precipitation most applicable to their waste. In this connection, some of the underlying fundamentals have been investigated at the Department of Bacteriology, University of Minnesota, and at their own laboratories in Austin.

In order to relieve pollution in the Cedar river as much as possible, the Hormel company, several years ago, provided for preliminary treatment such as the removal of the paunch manure by suitable screening, and the removal of easily settleable solids by primary sedimentation. Waste from the stock yards was collected and used as fertilizer on neighboring farms, and domestic sewage coming from the plant was put directly into the city sewers. These modifications, however, did not materially relieve pollution in the river. The by-passing of all condenser and other clean water served to reduce the volume of the sewage in preparation for chemical treatment. With these provisions, the Cedar river received about three-fourths million gallons of packing house waste that contained approximately 2500 parts per million of volatile solids and that had a B.O.D. of about 1800. From 75 to 80% of these solids were in colloidal suspension and could not be removed by further sedimentation. The balance appeared to be in true solution. The object of the investigation was to find ways and means of removing all of the former and as much of the latter as possible.

Various methods of chemical precipitation as a means of sewage clarification have been studied in the past, but none of them have been extensively applied in this country. In this connection there are various principles that

may be employed. The proteins may be precipitated by adjusting the pH to the isoelectric point, such as in the Miles Acid Process,¹ or they may be precipitated by neutralizing the charge with the salts of various metals, such as iron or aluminum. It may be argued that this is not exactly a charge neutralization, but that the protein salts of the heavy metals are formed. This is being investigated at this time, but results are not as yet ready for publication. Proteins may also be precipitated by denaturing them with oxidizing agents or other coagulants.

The Miles Acid Process was eliminated because good results can be obtained only in case the pH is very accurately controlled. This cannot be accomplished by manual methods in a plant that operates on a continuous flow, and since no fool-proof automatic methods have as yet been devised, it was felt that this process could not be relied upon for uniformly good results. Likewise, coagulation with aluminum or iron salts was rejected because it was felt that with manual control it would be difficult to avoid excessive ash content in the recovered product. Of the coagulants in the third class, chlorine appeared to be the most promising from an economic standpoint. That this chemical could be used for the precipitation of proteins was pointed out as early as 1840 by Mulder,² Thenard,³ Berzelius,⁴ and DeVrij.⁵ In 1897 Rideal and Stewart⁶ advocated the use of chlorine for the precipitation and quantitative determination of gelatin and peptone in meat extracts. The authors stated that the precipitate so obtained flocced and filtered readily and was quantitatively weighable. According to them, the percentage of gelatin found by this method checked very closely with that found by any other then in use. They also noted that the proper drying of the precipitate was an important factor, since at high temperatures the precipitate decomposed and became discolored. Their precipitation was accomplished by bubbling chlorine gas through the solution until coagulation was complete. It is apparent from this that they were using very large quantities of chlorine. In an article published in 1910 Rideal again points out that the chlorine will completely precipitate all proteins and peptones, but that it does not throw down the amino acid or organic bases even though it does combine with them. There is no indication in the literature that Rideal ever attempted to make use of this in a practical way for the treatment of sewage, although he does mention in his publication of 1910 that clarification observed when small amounts of chlorine were added to sewage might be due to this precipitation. This is in contradiction to his statement that when small quantities of chlorine are added, soluble compounds of the proteins are formed.

¹ U. S. Patent, 1,134,280. April 6, 1915.

² Berzelius *Jahresber.*, 19, 734 (1840); *Jahresber. Chem.*, 44, 489 (1848).

³ *Mém. d'Arcueil*, 2, 38. Quoted by Rideal.

⁴ *Jahresber.*, 19, 729 (1840).

⁵ *Ann. Pharm.*, 61, 288 (1847).

⁶ *Analyst*, 22, 228 (1897).

A great deal of work has been reported in the literature⁷ on the reaction of chlorine with proteins and protein products in connection with antiseptic studies. To the best of our knowledge, however, none of these references call attention to the protein-precipitating power of this element, and none of the investigators attempted to make any practical use of this property of chlorine. In fact, the reports in the literature would lead one to believe that the amounts of chlorine required are too excessive for any such purpose.

Our data show that native proteins are precipitated by comparatively small amounts of chlorine, while modified proteins such as gelatin require larger quantities, and peptones are precipitated only when very high concentrations are used. Our work confirms that of Rideal and other early investigators in showing that amino acids are not precipitated, although they may be reacted upon and sometimes decomposed by the chlorine. Our data also show that precipitation can be effected even in solutions that contain mixtures of various proteins and their decomposition products, although in such cases sufficient chlorine must be added to satisfy in part the demands of all the compounds present. In the following tables may be found the effects produced when the chlorine is added to pure solutions of various proteins and their decomposition products, as well as solutions containing mixtures of the two. The quantities of chlorine indicated in these data are approximately the minimum amounts required for the precipitation.

TABLE I

Effect of Chlorine on Various Proteins and Protein Derivatives

Substance	Nitrogen Grams per 100 cc	Chlorine Grams per 100 cc	Cl/N ratio	Remarks
Egg albumin	0.0096	0.0202	2.1	Precipitation-filtrate clear
Fresh blood	0.0096	0.0240	2.5	Precipitation-filtrate clear
Gelatin	0.0096	0.0403	4.2	No precipitate-filtrate milky
Gelatin	0.0096	0.0500	5.1	Partial precipitate-filtrate milky
Peptone	0.0096	0.0580	6.1	No precipitation
Peptone	0.0096	0.1530	16.0	No precipitation
Peptone	0.0096	0.3500	36.4	No precipitation-filtrate milky
Tryptophane	0.0096	—	—	No precipitation-filtrate red
Tryptophane	0.0096	0.1200	12.5	Precipitation-filtrate dark red
Glycine	0.0096	0.1920	20.0	No precipitation

⁷ Chattaway: *Trans. Chem. Soc.*, **87**, 145 (1905); **107**, 1814 (1915); Dakin: *Brit. Med. J.* Aug. 28, Oct. 23, Nov. 27, Dec. 4, 1915; (1) 852 (1916); Dakin, Cohen, Daufresne, and Kenyon: *Proc. Roy. Soc.*, **89 B**, 232 (1916); Dakin and Dunham: "Handbook on Antiseptics," (1917); Raper, Thompson and Cohen: *J. Chem. Soc.*, **85**, 371 (1904); Rideal; *J. Roy. Sanit. Inst.*, **31**, 33 (1910); Rideal and Rideal: "Chemical Disinfection and Sterilization" (1921); Smith, Drennan, Rettie and Campbell: *Brit. Med. J.*, (2) 129 (1915); Taylor and Austin: *J. Exp. Med.*, **27**, 155 (1899); Tilley: *J. Agr. Res.*, **20**, 85 (1920); Tilley and Chapin: *J. Bact.*, **19**, 295 (1930); Tonney and Greer: *Am. J. Publ. Hlth.*, **18**, 1259 (1928); Tonney, Greer and Liebig: *Am. J. Publ. Hlth.*, **20**, 503 (1930).

TABLE II

Effect of Chlorine on Mixtures of Proteins and Their Decomposition Products

Substances		Nitrogen		Chlorine	Cl/N ratio
A	B	Grams per 100 cc.		Grams per 100 cc	
		A	B		
Albumin	Tryptophane	0.0074	0.0000	0.0145	2.0
"	"	0.0070	0.0005	0.0240	3.2
"	"	0.0059	0.0020	0.0476	6.0
"	"	0.0037	0.0048	0.0817	9.6
"	Glycine	0.0074	0.0005	0.0215	2.8
"	"	0.0037	0.0048	0.0560	6.6
"	Peptone	0.0070	0.0005	0.0215	2.8
"	"	0.0037	0.0048	0.0301	3.5
"	Gelatin	0.0055	0.0024	0.0173	2.2
"	"	0.0037	0.0048	0.0173	2.1
Blood	"	0.0120	0.0048	0.0522	3.2
"	"	0.0180	0.0024	0.0522	2.5

It is to be observed that in the case of pure protein solutions, precipitation can be effected by comparatively small amounts of chlorine. The chlorine requirement is increased somewhat in the presence of peptones, but considerably more in the presence of amino acids, while gelatin increases the demand less than either of the former. Thus albumin and blood proteins are precipitated when the Cl/N ratio is 2.5 or less, whereas a ratio of 6.0 or above is required when amino acids are present. Since the Cl/N ratio is calculated from the total nitrogen present, it is apparent that nitrogen compounds which are not precipitated will lower the efficiency of the process to an even greater extent than is indicated by the ratios given in the above table. Table III further emphasizes this fact by showing that the percentage removal decreases materially when amino acids or peptones are present.

TABLE III

The Percentage Removal of Nitrogen by Chlorine Precipitation of Various Organic Nitrogen Mixtures

Substance		Concentration		Grams N in precipitate	Percentage removal
A	B	Gms N per 100 cc			
		A	B		
Albumin		0.0418		0.0417	99.7
Albumin	Gelatin	0.0208	0.0272	0.0441	91.8
Albumin	Blood	0.0208	0.0105	0.0303	96.8
Albumin	Tryptophane	0.0208	0.0099	0.0220	71.6
Albumin	Peptone	0.0208	0.0254	0.0209	45.2

In the case of mixtures of gelatin and protein, it appears that the gelatin is precipitated even though the Cl/N ratio is less than that ordinarily required to precipitate it alone. Thus we see that in Table III where a nitrogen

removal of 91.8 is obtained, a considerable portion of the nitrogen must have come from the gelatin. The flocculent precipitate formed by the native proteins apparently occludes the fine colloidal precipitate formed from the gelatin, so that a clear filtrate is produced in a mixture of this type, whereas in a pure solution of gelatin the fine precipitate will not settle out. To produce a clear filtrate with a mixture of native proteins and gelatin, it is necessary to stir the solution gently for about 10 minutes following the addition of the chlorine.

In coagulating proteins, definite ranges of chlorine concentration are required before any precipitate is formed. Small amounts of chlorine do not produce proportionate amounts of precipitate, but instead all the proteins precipitate when a definite range is reached. This is illustrated in the following table which shows the results obtained with different concentrations of egg albumin.

TABLE IV

The Effect of varying the Chlorine Concentration on Albumin Solutions

Concn. egg albumin Gms. N per 100 cc.	Concn. Chlorine			Cl/N ratio	
	No. pptn.	Pptn. starting	Pptn. complete	Pptn. starting	Pptn. complete
0.074	0.145	0.160	0.170	2.1	2.3
0.0074	0.016	0.0165	0.0170	2.2	2.3
0.00148	0.0029	0.0032	0.0034	2.1	2.3
0.00074	0.0012	0.0017	0.0017	1.9	2.3

The above data indicate that definite proportion of chlorine to nitrogen is needed before precipitation occurs, regardless of the concentration of the latter. This would imply that the chlorine requirements are independent of the concentration of organic matter. This has been checked by determining the minimum amount of chlorine required to produce precipitation after a reaction period of 15 minutes. The data are given in Table V.

TABLE V

The Effect of Concentration of Protein on the Cl/N Ratio

Concn. of Egg Albumin Gms. N in 100 cc.	Concn. of Chlorine Gms. per 100 cc. necessary for precipitation	Cl/N ratio
0.1000	0.2250	2.25
0.0500	0.1125	2.25
0.0250	0.0580	2.28
0.0200	0.0436	2.18
0.0100	0.0224	2.24
0.0050	0.0102	2.04
0.0025	0.0051	2.04
0.0011	0.0019	1.80
0.0001	No visible precipitate	

Within experimental error, it appears that the quantity of chlorine required is dependent only upon the amount of protein present and independent of its concentration. The slight decrease which occurs in the dilute solution may be due to experimental error which is difficult to avoid in those cases.

The chlorine-nitrogen ratio of from 2.0 to 2.3, which is necessary for the precipitation of proteins from pure solutions, can be lowered considerably by an adjustment of the reaction. In pure solutions the final reaction is brought to a point somewhere between pH 2.0 and 4.0, depending upon the concentration of proteins present. In reacting with the protein, the major portion of the chlorine is converted to hydrochloric acid, which causes a lowering in the pH. If some of this acid is neutralized so that the final pH is about 4.0, precipitation can be effected with considerably less chlorine. That it does not appear to make any difference which alkali is used for this purpose is indicated in the table below. The same end result is obtained whether the alkali or chlorine is added first. The amount of alkali needed depends upon the quantity of chlorine used, and since that in turn is governed by the concentration of protein present, the alkali dosage must be varied in accordance with the strength of the solution treated. In samples of waste from a packing establishment, we have found that there is usually present more than enough alkali in the form of carbonates of calcium and sodium. In those cases, then, the pH must be adjusted to 4.0 by adding a small amount of mineral acid or by adding a little excess of chlorine. The condition can be partially alleviated by preventing clean waters, which usually contain carbonates, from being mixed with the sewage. Tables VI and VII show the minimum amounts of chlorine which give a clear supernatant liquor when the reaction is adjusted to the optimum.

TABLE VI

The Effect of Various Alkalies on the Chlorine Demand
in Protein Precipitation

Concn. Protein Gms. N per cc.	Minimum gms. chlorine required to precipitate	Cl/N ratio	Comparative minimum amounts of alkali required for adjustment		
			N/130 CaO	N/10 NaOH	N/10 Na ₂ CO ₃
0.0077	0.0090	1.1	20 cc.	1.4 cc.	1.4 cc.
0.0134	0.0157	1.1	35	2.5	2.7
0.0192	0.0225	1.1	50	3.7	3.6

In Table V above it was shown that for a certain range the Cl/N ratio is independent of the concentration of the protein. The same condition is true if the reaction is adjusted as shown in Table VIII.

Here, as in the unadjusted series, the Cl/N ratio appears to be independent of the amount of protein present, particularly in the more concentrated solutions. There appears to be a slight increase in the ratio in dilute solutions, although this may be due to experimental error. The percentage error will naturally be high in the solutions that contain 25 p.p.m. or less of nitrogen.

TABLE VII

The Effect of Alkali on the Chlorine Precipitation of Various Proteins

Protein	Nitrogen Grams per 100 cc	Alkali	Chlorine Grams per 100 cc	Cl/N ratio
Albumin	0.0096	None	0.0210	2.1
"	0.0096	CaO	0.0130	1.3
Gelatin	0.0096	None	0.0420	4.2
"	0.0096	CaO	0.0210	2.1
50-50 mixture albumin and gelatin	0.0096	None	0.0260	2.6
" "	0.0096	CaO	0.0150	1.5
50-50 mixture gelatin and blood	0.0096	None	0.0310	3.2
" "	0.0096	CaO	0.0100	1.0
50-50 mixture albumin and peptone	0.0096	None	0.0240	2.5
" "	0.0096	CaO	0.0125	1.3
50-50 mixture blood and albumin	0.0096	None	0.0280	2.8
" "	0.0096	NaOH	0.0110	1.1
" "	0.0096	CaO	0.0110	1.1
Blood	0.0096	NaOH	0.0105	1.1
"	0.0096	None	0.0270	2.8
$\frac{1}{3}$ each albumin, blood and peptone	0.0096	None	0.0480	5.0
" "	0.0096	CaO	0.0270	2.7

TABLE VIII

The Effect on Chlorine Consumption of varying the Protein Concentration with an Adjusted Reaction

Concn. Egg Albumin Gms. N per 100 cc.	Concn. Chlorine necessary for precipitation Grams per 100 cc.	Cl/N ratio
0.1000	0.1170	1.17
0.0500	0.0560	1.12
0.0250	0.0285	1.14
0.0200	0.0218	1.09
0.0100	0.0109	1.09
0.0050	0.0057	1.14
0.0025	0.0031	1.24
0.0010	0.0014	1.40
0.0001	No visible precipitate	

Since the Cl/N ratio is constant in solutions containing 100 p.p.m. or more of nitrogen, it is possible to predict the nature of the curve that would be obtained if this ratio were plotted against protein concentrations. This is illustrated in Fig. 1. The solid lines are plotted from the experimental data given in Tables VII and VIII. The dotted lines are predicted. It is assumed that with concentrations above 0.1000 the Cl/N ratio will remain constant. Since the amount of HCl generated decreases with a decrease in nitrogen content, the amount formed when the solution contains less than 25 p.p.m. of nitrogen will be only slightly greater than that required to adjust

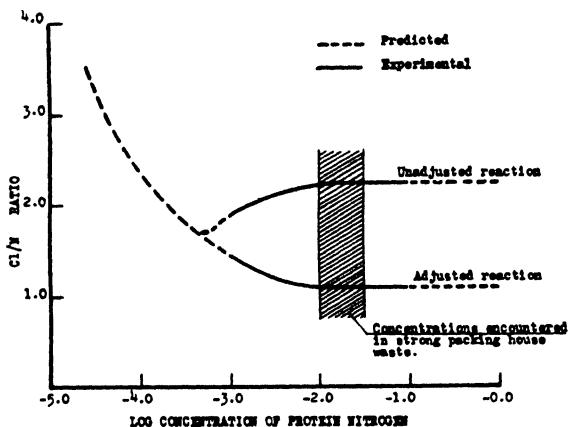


FIG. 1

Effect of Chlorine Concentration on The Chlorine-Nitrogen Ratio

the reaction to its optimum. Since conditions are then more favorable for precipitation, less chlorine should be required. This accounts for the drop in the curve for the unadjusted reaction. This tendency will continue until the chlorine added is the exact amount for the pH adjustment. After that, additional chlorine must be used to acidify the solution, and the Cl/N ratio will naturally increase.

As the reactions in the unadjusted solutions approach the optimum, less alkali will be needed to neutralize the excess acid formed, until finally none at all will be needed. At this point, then, no saving in chlorine will be effected by the addition of alkali, and the two curves will meet. Since the points in the above curve that show deviations from a straight line occur with dilute solutions in which experimental errors are large, the entire curve should be regarded as theoretical until verified by more rigid experimental data. The shaded area shown in Fig. 1 represents nitrogen concentrations of between 100 and 300 p.p.m., which strength can be easily obtained in packing plant wastes. In this range the Cl/N ratio is independent of strength. If the strength is reduced below 100 p.p.m. of nitrogen, the ratio for the adjusted series increases with increasing dilution. Since normal packing house sewage usually contains an excess of alkali, this is the curve which will normally be followed. In case there should be no alkali present, it will be desirable, from

the standpoint of economy, to add some. The adjusted curve shows the desirability of concentrating the sewage to a point where it will contain 100 p.p.m. or more of organic nitrogen. This is generally accomplished by bypassing the clean water coming from the plant. Since such waters contain bicarbonates of both sodium and calcium, their elimination has the additional advantage of reducing the excess alkalinity.

In addition to the optimum that exists at approximately pH 4.0, there appears to be another optimum at pH 2.0 or less, particularly with chlorinated egg albumin. Thus we find that the Cl/N ratio can be decreased to 1.0 if a comparatively large amount of H_2SO_4 is added. This range has not been

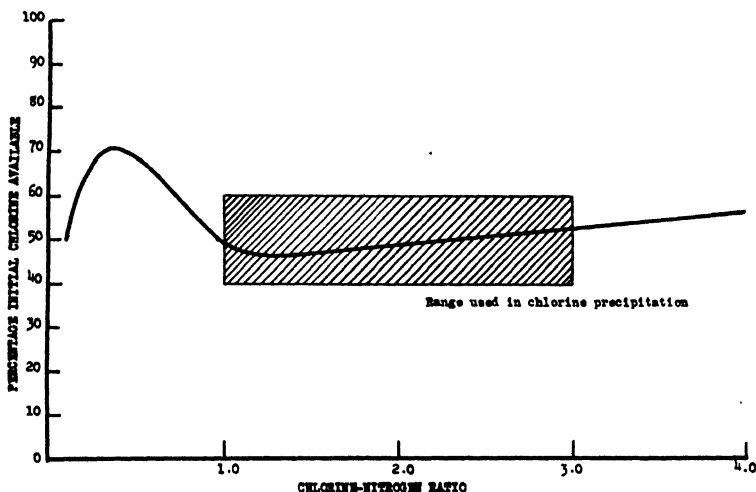


FIG. 2

Percentage of Initial Chlorine available in Various Mixtures of Chlorine and Gelatin.
(Data taken from N. C. Wright)

investigated with other proteins, since the large amount of acid necessary would counteract any saving of chlorine that could be effected. In addition, other undesirable conditions would be encountered.

In the reaction between chlorine and protein, a portion of the chlorine reacts with the amino groups to form chloramines, a portion may replace hydrogen in other parts of the molecule, some may react into double bonds, and another portion, in the form of hypochlorous acid, may react in such a way that oxygen is introduced into the molecule instead of chlorine. In this connection, and in the light of the researches of Dakin⁸ and Wright,⁹ our data are of particular interest.

Dakin has shown that the percentage of chlorine forming chloramines depends upon the relative amounts of protein and chlorine taking part in the reaction. This was investigated in more detail by Wright. He confirmed the

⁸ Dakin: *Biochem. J.*, 11, 79 (1917); 10, 319 (1916); *Proc. Roy. Soc.*, 89B, 232 (1916); *Brit. Med. J.*, 1, 852 (1916).

⁹ Wright: *Biochem. J.*, 20, 525 (1926) and Personal Communication.

results of Dakin and was able to show graphically the amount of available chlorine present when varying quantities of proteins were added to fixed amounts of chlorine. From his data, we have been able to correlate the amount of available chlorine with the Cl/N ratio. This has been done for gelatin, and is illustrated in Fig. 2. In a private communication, Wright has presented data which show that the position and shape of the curve varies with the pH, so that the relationship shown in Fig. 2 should not be regarded as definite for all pH values.

It can be observed from this curve that when chlorine is added to a protein, a considerable amount can no longer be accounted for by thiosulphate titration. With a chlorine nitrogen ratio of from 1 to 5, as shown by the shaded area in Fig. 2, more than 50% of the chlorine is unavailable. If we assume that all of this has reacted as an oxidizing agent, it is possible, from determinations of available chlorine in the supernatant liquor and precipitate, to show the percentage that has reacted in this way. The data are presented in Table IX.

It is to be observed from these data that from 48 to 64% of the chlorine becomes unavailable when no alkali is used for the adjustment of the pH. With the alkali present, the unavailable chlorine ranges from 72 to 92%. Attempts have been made to determine the exact amount of this which has reacted in the form of hypochlorous acid with the introduction of oxygen into the protein molecule. Suggestive data have been obtained, but further work is required before definite conclusions can be drawn. By titrating with standard alkali, the total acidity has been determined. If this is calculated on the basis of HCl, we have found that its chlorine equivalent checks, within experimental error, with the unavailable chlorine plus one-half of the available. Since, when chlorine reacts with the amino groups to form chloramines, one-half of the chlorine goes to HCl, the results indicate that all of the unavailable chlorine has reacted as an oxidizing agent introducing oxygen into the protein molecule. Since the method used for determining the amount of HCl formed is not very accurate, particularly in the presence of protein buffers, it cannot be stated definitely that no chlorine is introduced into the protein molecule except that which is in the form of chloramines. It should, however, be safe to conclude that the majority of the unavailable chlorine has been converted to HCl. We have also observed that the modified proteins are precipitated at pH 4 or less as readily when the available chlorine has been removed by thiosulfate as when the chloramines are left intact. This would lead one to believe that the chlorine which has reacted as an oxidizing agent is of primary importance in modifying the proteins for the precipitation.

It is of particular interest to note that the amount of available chlorine in the supernatant liquor is very low when native proteins are the only nitrogenous compounds present, and particularly so when the reaction is adjusted. The amount increases in proportion to the concentration of nitrogenous material that is not precipitated. In the practical application, the available chlorine in the effluent ranges from 25 to 50 parts per million.

TABLE IX

Distribution of Available Chlorine in Protein Precipitation

Composition	Total N (Grams)	Available Chlorine		Gms. Cl. added	Percentage Cl. used in oxidation
		P.p.m. in effluent	Grams in ppt. from 100 cc. solution		
100 parts albumin, no alkali	.0077	.0016	.0033	.0126	61
	.0077	.0003*	.0052	.0151	64
	.0077	.0008	.0061	.0063	58
75 parts albumin	.0077	.0047	No. ppt.	.0126	63
25 parts peptone, no alkali	.0077	.0028*	.0032	.0151	60
	.0077	.0026	.0052	.0163	48
50 parts albumin	.0077	.0051	.0016	.0150	56
50 parts peptone, no alkali	.0077	.0048*	.0017	.0163	60
	.0077	.0047	.0029	.0201	63
25 parts albumin,	.0077	.0066*	.0012	.0163	52
75 parts peptone,	.0077	.0066	.0016	.0201	59
no alkali	.0077	.0069	.0018	.0227	62
50 parts albumin,	.0077	.0006	.0002	.0050	84
50 parts peptone,	.0077	.0012*	.0005	.0075	78
with alkali	.0077	.0020	.0008	.0100	72
100 parts albumin,	.0077	.0002	.0006	.0050	84
with alkali	.0077	.0002*	.0010	.0062	81
	.0077	.0002	.0013	.0075	80
Albumin with no alkali	.0192	.0012*	.0130	.0375	62
Albumin with alkali	.0192	.0000*	.0015	.0175	92
50 parts albumin					
50 parts peptone, no alkali	.0192	.0124*	.0067	.0450	58
50 parts albumin					
50 parts peptone, with alkali	.0192	.0025*	.0017	.0200	79

* Combinations in which the minimum amount of chlorine was used to give complete precipitation.

The above theoretical studies are of value in determining the limitations of the process. For efficient and economical chlorine precipitation, the waste must contain a comparatively large amount of native proteins, and must be treated before extensive septic action has taken place. From our experience with the waste from the plant of Geo. A. Hormel & Co., we feel that packing

house sewage can be successfully treated by chlorine precipitation. We have found that from various samples of waste from the Hormel plant, 40 to 85% of the organic nitrogen can be removed. Samples containing relatively high percentages of blood gave the higher results, whereas those that had become septic or otherwise contained large amounts of peptone or gelatin gave lower yields. Composite samples from typical runs usually showed removals of from 60-89%. The following table presents some representative results of actual plant operation.

TABLE X

Substance	Data on Plant Operation		Percentage Reduction
	Raw Sewage (parts per million)	Effluent	
Organic nitrogen	166.84	44.0	73.0
Ammonia nitrogen	17.31	14.4	—
Total nitrogen	184.15	58.4	68.0
Total solids	4971.00	3235.0	35.0
Fixed solids	2431.00	2582.5	—
Volatile solids	2540.00	652.5	74.4
Sodium chloride	2422.00	2292.0	—

10,591 pounds of sludge obtained from 660,000 gallons.

The data reported in the above table are representative of data obtained from carefully composited samples on days when the packing plant was operating at normal capacity. The significant figures are those showing reduction in organic nitrogen and volatile solids, the former being 73% and the latter 74%. In the various analyses that have been made, these figures show a variation of from 60 to 80%.

When the plant is operating at normal capacity about 5 tons of sludge are obtained daily. The above figure of 10,591 pounds is representative. About two tons of this are obtained in a primary clarifier without chlorine precipitation, while the other three tons are obtained in a secondary clarifier following the introduction of enough chlorine to give a Cl/N ratio of from 1.3 to 1.5. The following is a representative analysis of the sludge obtained by chlorine precipitation after it has been dewatered and dried.

Moisture.....	3.70%
Protein.....	48.90%
Ammonia.....	9.50%
Fat.....	22.72%
Ash.....	11.60%

The engineering features and practical results of the plant, having been published elsewhere,^{10,11} are not repeated here for the sake of brevity. As has been pointed out in previous publications, the cost of installation is about one-third of the estimated cost of a biological plant. The saving in depreciation and

¹⁰ Municipal Sanitation, April 1931.

¹¹ Sewage Works Journal, 3, No. 3, 488 July (1931).

interest on installation cost is more than enough to pay for chlorine at present prices. Since other operating costs are low, even if the recovered sludge be considered of no value, the process compares favorably from an economic standpoint with any other method that has been devised for the treatment of packing plant wastes.

The ultimate value and use for the sludge is problematic. On feeding the dry sludge to rats we found that it was toxic. Young rats died in four days when fed the dry sludge as the sole source of nitrogen. By extracting the sludge with ether or petroleum ether, the toxicity was reduced to a point where it was doubtful whether the effects produced were due to toxic materials or to the lack of certain essential amino acids. On feeding the extracted material to hogs along with corn no ill effects could be observed, and the animals showed a greater gain in weight than controls getting corn only. These preliminary results warrant further investigation, the results of which may show that the precipitated protein can be rendered fit for hog food. In case this cannot be done, it is felt that the sludge will have real value as nitrogen fertilizer. Experiments are now in progress to determine its value in this respect.

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THE STRUCTURE OF RUBBER AND OTHER ELASTIC COLLOIDS

BY G. S. WHITBY

Efforts have been made in recent years to ascertain the structure of rubber and explain its properties, by consideration particularly of (a) the structure of the globules in rubber latex, (b) the behaviour of rubber towards swelling agents (especially ether), (c) the X-ray diagram given by rubber under certain conditions. The purpose of the present paper is to consider current views concerning the structure of rubber, and also to point out that, since other colloids are known which exhibit in greater or less degree elastic properties similar to those exhibited by rubber, any general view of the structure of rubber can—it must be assumed, unless cogent reasons to the contrary are adduced—be regarded as acceptable only if it is also applicable to such other colloids. Its purpose is, further, to suggest that a study of such other elastic colloids is helpful in elucidating the structure of rubber.

A microscopical study (using the micromanipulator) of the globules in *Hevea* latex led Freundlich and Hauser¹ to conclude that the globules consist of rubber in two distinct forms: that they are composed of “a viscous liquid surrounded by an elastic shell”.² The inner portion of the globule was considered as being caoutchouc in a lower and the outer as caoutchouc in a higher state of polymerization. The two forms of rubber hydrocarbon which these authors believed to be recognizable in the globules were at first assumed³ to be identical with the parts, sol and gel, obtainable from raw rubber by the ether diffusion process (*vide infra*), but more recently Hauser has apparently modified this opinion.⁴

Von Weimarn⁵ has concluded that the microscopical phenomena presented by rubber latex cannot be interpreted as proving the presence of a solid shell of rubber on the outside of the globules; and the present writer's observations, using the micromanipulator, are in agreement with this conclusion.

Leaving aside the question of the structure of the globules in rubber latex, a two-phase structure of a lower order, not recognizable by microscopic examination, has been considered to be present in rubber by many recent writers and has been made the basis of explanations of many of the properties of rubber. The two phases in question are usually known as sol and gel rubber and are distinguished by the fact that they are respectively soluble and insoluble in ether.

In point of fact the idea that raw rubber consists of two parts, identical in proximate composition, but one soluble and the other insoluble, is an old idea which has been lately revived and made the basis of explanations of some of the more striking modern phenomena observed with rubber, especially by means of X-rays. The idea was expressed by Payen,⁶ Herbst,⁷ Gladstone and Hibbert⁸ and Weber.⁹ Caspari,¹⁰ by treating raw rubber with petroleic

ether, separated it into a soluble and an insoluble portion, the latter of which he referred to as the "pectous" form of rubber. He believed that it was possible to make a definite separation of rubber into the two forms, and that the proportions in which they occurred varied from sample to sample, and could be determined quantitatively. Stevens¹¹ reported, however, that, on repeating Caspari's experiments, he had been unable to obtain concordant results in repeat experiments. "The proportion of soluble to 'pectous' appeared to depend on the period of extraction." "Moreover," he said, "I find that the 'pectous fraction', if allowed to stand sufficiently long in cold petroleum spirit, dissolved wholly, with the exception of a small quantity of slimy nitrogenous matter which settled to the bottom of the containing vessel."

In recent years Caspari's views concerning the twofold nature of rubber have been revived; in the first place by Feuchter,¹² who applied ethyl ether instead of petroleic ether for the separation of the two parts. The portion of raw rubber which diffuses into ether when the rubber is allowed to stand in that liquid, and which corresponds to Caspari's "soluble" rubber, Feuchter designated Diffusion or D-rubber, while the portion which remains, and which corresponds to Caspari's "pectous" rubber, he designated as the gel skeleton. These two parts are now commonly known as sol and gel rubber, respectively, although Hauser² prefers the terms alpha- and beta-rubber. According to present usage, sol rubber is such portion of raw rubber as diffuses from the swollen mass into the solvent when rubber is allowed to stand in ethyl ether, whereas gel rubber is such portion as remains behind.

The view that rubber consists of two parts sharply separable by means of ether has been much in the foreground in recent years. Several writers,¹³ among whom Hauser is prominent, have seen in the presence of sol and gel portions in rubber an explanation of the X-ray diffraction phenomena which rubber shows when stretched. It has also been called upon to explain the behaviour of rubber on mastication,¹⁴ the Joule effect,¹⁵ and the elastic behaviour of rubber generally; and, further, has been made the basis of an explanation of vulcanization.¹⁶

Although in its normal unstretched condition, raw rubber, when examined by X-rays, gives only an amorphous ring, it gives a fiber diagram with X-rays when strained beyond about 80 per cent.¹⁷ If the amount of strain is gradually increased to say 1000 per cent, the interference spots on the X-ray diagram gradually increase in intensity but do not change in position. It has been supposed that the appearance of a diffraction pattern on stretching rubber is due to the occurrence of a "de-swelling" of the gel by the sol phase. Such a de-swelling seems to the present writer improbable. Assuming that the phases are to be regarded as imbibed each by the other, they are in the normal condition swollen to an extent far short of their maximum capacity for swelling, and it seems unlikely that mere extension would produce a syneresis. Elastic colloids (e.g. vulcanized rubber), after being caused to imbibe ten or more times their own weight of a liquid, can be stretched without any apparent occurrence of syneresis. And it does not seem likely that a syneresis on

stretching would occur in raw rubber, where, on the view under consideration, the higher polymeric phases are swollen only slightly and where the "swelling agent" is itself a solid, namely the lower polymeric phase.

If raw rubber is kept for a considerable time at a low temperature, until it becomes "frozen," it then gives with X-rays a Debye-Scherrer diagram in the unstrained condition. This has been attributed to an increase in the gel component at the expense of the sol component during the period of storage at a reduced temperature. Conversely, it has been supposed that on subjecting rubber to heat or mechanical working a change of gel to sol takes place, and that on keeping rubber which has been so treated the change gradually reverses itself, the rubber tending to regain its original properties.¹⁸

X-ray studies of stretched elastic colloids cannot yet be said to have solved the problem of the cause of elasticity in such materials. It is true that raw rubber, when stretched to moderate elongations, gives an X-ray diffraction pattern, which indicates that certain parts of the material have assumed a definite configuration, but vulcanized rubber, which has better elastic properties, requires to be stretched much further before it gives a pattern, while polyvinyl acetate shows no pattern at 1500 per cent elongation and polystyrene none at 1300 per cent elongation.¹⁹ It would seem that the regular orientation of parts which an X-ray diffusion pattern connotes is not essential to the possession of elastic properties. Indeed, the occurrence of such a pattern is rather to be regarded as indicating that the material is assuming the character of an inelastic fiber.

The assumption of a fibrous character by rubber when it is stretched can be readily demonstrated, as Hock²⁰ showed, by cooling stretched rubber until it becomes brittle and shattering it by a blow. Other writers²¹ have shown that when rubber is stretched and then cooled until almost inextensible, the tensile strength is higher the greater the original stretch. Thus Mark and Valko²² found that a sample of raw rubber which had a tensile strength of 360 kg./cm.² at 195°, had a tensile strength of 2470 kg./cm.² if stretched 700% before cooling. This is in accord with the general property of fibers, first demonstrated by Herzog²³ for rayon, that the greater the degree of orientation of the elements of the fiber, the greater is the tensile strength.

Experimental evidence, some of which will be outlined in what follows, clearly shows that rubber and other elastic colloids are highly heterogeneous. Hence, in the opinion of the present writer, the view that rubber consists of two parts, "sol" and "gel," represents far too great an initial simplification of the issues involved in describing the nature of rubber and explaining its elastic behaviour. In the writer's view rubber does not consist of caoutchouc in merely two states of polymerization but is a mixture of an unbroken series of polymers representing a rather wide range of degrees of polymerization.

That the X-ray diffraction pattern given by stretched rubber is not due to the presence of two forms of rubber, one diffusible into swelling agents and the other not thus diffusible, is shown by the fact that diffusion-caoutchouc itself gives a pattern. A sample of diffusion-caoutchouc, prepared by removing the resin from raw rubber by cold extraction with a petroleic ether-

acetone mixture (3:7) and then allowing the rubber to stand in benzene until a portion of it had diffused into the solvent, was examined at extensions of 300% and 1800%. At both it gave a diffraction diagram, the diagram at the latter extension being remarkably sharp.²⁴

In other respects too, diffusion-caoutchouc shows all the normal behaviour of rubber. Naturally, its behaviour is not quantitatively identical with that of the original rubber from which it is obtained, since it represents only a fraction of the latter and the range of polymeric states in it is not identical with that in the original rubber. It can, however, be vulcanized, especially if small proportions of fatty acids are added to replace those removed in freeing the rubber from "resin" before diffusion; it shows "racking" phenomena (cf. *infra*); it requires breaking down on a mill in making up a stock, and the ease of breakdown is not strikingly different from that of the original rubber. The last-mentioned observation is opposed to the attempts which have been made to describe the phenomena involved in the mastication of rubber on the basis of its supposed dual nature. The fact that rubber must be "broken down" by mastication on a hot mill in order to render it plastic has been ascribed to the necessity of rupturing the shells supposed to surround the liquid centres of the latex globules or more generally to producing a dispersion of the gel rubber in a continuous medium of sol rubber.

If the diffusion of a portion of raw rubber into a solvent is allowed to proceed only a short way, the diffusion-caoutchouc isolated is still an elastic solid substantially similar in physical consistency to the original rubber. If there were a liquid phase in rubber, it would be expected that this would appear first on extraction. The fractional extraction of rubber affords, however, no indication of the presence of a liquid phase.

A study of the behaviour of rubber towards solvents²⁵ clearly indicates that the degree of heterogeneity of rubber is much greater than that suggested by the sol-gel conception. The latter conception, as already stated, came to the fore as a result of Feuchter's separation in 1925 of rubber into a portion soluble and a portion insoluble in ether. Experiments started by the writer in 1923 on the behaviour of rubber in some 400 organic liquids show that rubber cannot be separated sharply into a soluble and an insoluble part, but that the portion which passes into solution depends greatly on the nature of the liquid employed, the length of time over which the solution or diffusion process is allowed to proceed, and other circumstances. It was early observed that when weighed pieces of raw rubber are placed in organic liquids, and the imbibition followed by weighing the pieces at intervals, in a large number of cases the weight at first rises and the later falls. That the fall is due to diffusion of part of the rubber from the swollen mass into the liquid can easily be shown by adding alcohol or other precipitant to the latter, when the diffused rubber is thrown out. The process of diffusion of rubber from the swollen gel goes on gradually over a long period and does not apparently ever come to a sharp end. Some of the samples were kept under observation for four years, and in many such cases the whole of the rubber with the exception of the protein ultimately became dispersed in the liquid. Even in relatively poor swelling

agents (see, e.g., o-tolualdehyde, octylene, isoamyl oxalate, chloracetal, cyclohexanone in Table I), the dispersion process was often observed to have completed itself after 3-4 years. The general impression made by the mass of data which has been secured on the swelling of raw rubber is that in general the process of diffusion never comes to a definite end or allows of a sharp separation of the rubber into a "sol" and a "gel" portion. A little of the data is quoted in Table I.

TABLE I

Imbibition of Organic Liquids by Smoked Sheet (Original Weight: 1)

Liquid	Weight after periods of days						4 years
	1	2	3	7	31	62	
Octylene	6.68	4.46	4.22	3.31	—	—	Completely dispersed.
o-Tolualdehyde	3.67	4.66	4.88	4.00	1.65	—	"
Chloracetal	5.99	5.57	4.71	2.50	1.18	—	"
Cyclohexanone	6.14	4.84	2.83	1.40	—	—	"
n-Valeric anhydride	1.84	2.44	2.95	3.39	—	—	"
Isoamyl oxalate	1.51	2.13	2.76	3.40	—	—	"
Ethyl-m-toluate	7.98	8.39	8.25	6.07	2.92	2.36	
Isobutyl acetate	9.00	8.90	6.96	3.10	1.61	—	
p-Tolualdehyde	2.83	3.59	4.15	4.24	2.41	0.87	
o-Tolunitrile	5.67	7.10	7.79	7.43	4.82	2.43	
n-Propyl ether	5.98	5.25	4.29	3.03	2.27	1.87	
Safrole	9.51	11.60	12.01	10.36	5.86	4.51	
p-Cresyl ethyl ether	13.18	14.85	13.16	9.02	5.66	4.81	
Butyrophenone	4.83	5.95	6.18	4.30	—	1.60	
Allyl isothiocyanate	7.44	9.60	9.65	5.63	2.82	—	
Lauryl chloride	7.49	8.52	7.86	5.76	3.02	—	
Phenyl ethyl bromide	7.23	9.03	9.26	5.36	2.91	—	
Nonylic acid	3.37	5.00	6.50	6.91	1.58	—	
Tripropylamine	9.50	7.02	5.45	4.09	2.85	2.12	

The examples quoted in Table I have been chosen to represent liquids belonging to a considerable number of different chemical types. The figures show the weight of the swollen mass after various periods of time, the initial weight being taken as 1. In some but not all cases the samples were examined after a lapse of four years. Most of the liquids are not among the most powerful swelling agents. With such powerful swelling agents the swollen mass is usually too weak to handle for the purpose of weighing after one day. It may be mentioned for purposes of comparison that the weight of e.g. benzene imbibed in one day by the specimen of rubber in question was 24.22 parts; of ether, 11.10 parts.

If the point at which the process of diffusion becomes very slow were to be taken as the end point, the proportions of "sol" and "gel" which it would be concluded that the rubber contained would undoubtedly vary greatly with the solvent used.

Although there is no sharp end to the diffusion process, the ease with which diffusion occurs and the proportion which will diffuse into the solvent with reasonable rapidity, vary in different liquids, and are apparently influenced by (a) the swelling power of the liquid, (b) its viscosity. Even in a given liquid the proportion of caoutchouc which will diffuse in a reasonable time from a given sample of rubber can be greatly modified. Such modification can be brought about by (among various means) adding small quantities of certain agents, especially strong organic bases and acids, which increase the amount of the liquid imbibed by the rubber. When studying the electro-viscous effect in rubber sols in 1924, the author employed diffusion into ether and petrolic ether for the purpose of securing acoutchouc free from protein and observed that the addition of small amounts of piperidine, diethylamine, sodium ethoxide and ammonia greatly increased the proportion of diffused caoutchouc obtained from a given sample of raw rubber. Previously it had been found that small amounts of piperidine increase the swelling of vulcanised rubber in benzene.²⁶

The influence of bases on the swelling and dispersion of raw rubber may be illustrated by the following data:—

Smoked Sheet in Petrolic Ether (B.P. 25-45°).

	A	
	Pet. ether alone	Pet. ether containing 5 drops piperidine per 10 ccs.
Weight originally	0.1317 grms.	0.1075
Weight after swelling 24 hours	1.0925 grms.	1.7435 grms.
Increase in weight	735 per cent	1520 per cent

	B	
	50 ccs. pet. ether	40 ccs. pet. ether + 10 ccs. ethereal solution NH ₃
Weight originally	0.60 grms.	0.60 grms.
Weight of diffused caoutchouc pptable after 8 days' standing	0.05 grms.	0.31 grms.

In a good swelling agent such as benzene the proportion of caoutchouc which will readily diffuse from a sample of smoked sheet is greater than in a poorer swelling agent such as ether or especially petrolic ether. Thus, for example, in an experiment in which 3.83 grms. smoked sheet was left in 190 ccs. benzene with occasional very gentle agitation, 75.5 per cent of the rubber has dispersed in 7 days.

A study of the behaviour of vulcanized rubber in swelling agents also fails to afford any evidence of the presence of two sharply distinct parts. In current writings on the structure of rubber less attention has been given to vulcanized than to raw rubber, although the elastic properties which any

theory of the structure of rubber must ultimately endeavour to explain are exhibited in much greater perfection by the vulcanized than by raw material. It, however, the X-ray diffraction phenomena shown by raw rubber are ascribed, as they currently are, to its two-fold (sol-gel) nature, then it is natural to assume that, since vulcanized rubber shows similar X-ray phenomena, it too consists of two parts, although the proportion in which the two parts occur may be regarded as different, owing to the conversion during vulcanization of a portion of the sol into the gel form. (Cf. Hauser: *Trans. Inst. Rubber Ind.*, 1926, p. 243:—"During vulcanization the liquid rubber phase is more or less gelled or polymerised.")

Observations on the behaviour of vulcanized rubber in swelling media show, as in the case of raw rubber, no evidence of the presence of two clearly distinct parts. It is usually said that vulcanized, in contradistinction to raw rubber, is insoluble in all liquids. The writer has, however, found that, by leaving fully-vulcanized rubber in swelling agents for periods up to four years in the dark, the rubber gradually dispersed in many cases. Further, the rubber dispersed completely or substantially so, and there was no indication of the presence of a soluble and an insoluble phase. Examples of liquids in which vulcanized rubber was observed to disperse completely in the course of time are ethyl benzoate, butyl acetate, butyl oxalate, ethylene glycol dipropionate and twenty-one other esters; methyl iodide, bromoform, isobutyl chloride, o-chlorotoluene and twelve other halogenated hydrocarbons; benzene, valeric acid, ethyl propyl ketone, cyclohexanone, butyl isothiocyanate, hexyl alcohol, and o-tolualdehyde, diisobutylamine, n-propyl ether.*

A number of artificial colloids which show elastic and colloid properties fundamentally similar to those of raw rubber can readily be shown to be highly heterogeneous. Such materials, although less important industrially than rubber, are more tractable than rubber and a study of them is capable of throwing light on the structure and behaviour of rubber, not only in regard to the question of heterogeneity but also in other respects.

Examples of artificial elastic organic colloids are synthetic caoutchoucs from conjugated dienes of low molecular weight, reaction-products from fatty oils and sulphur, polymers of methyl acrylate and similar esters, polymers of styrene, vinyl acetate and ethyl itaconate, caoutchouc hydrochloride, the reaction product from isoprene and sulphur dioxide. The first three of these examples are elastic at room temperature; the others, when warmed or swollen. Of these materials polystyrene and poly-vinyl acetate have been most closely investigated.

Polystyrene at ordinary temperatures is a hard solid; clear, when in massive pieces or fibers; white, when precipitated. At ordinary temperature it is not elastic, the films being brittle and the powder friable, but, as was first observed by the writer,²⁷ when warmed or swollen, it shows elastic properties. The

* In this connection it may be mentioned that the viscosity of such sols of vulcanized rubber is very much less than that of sols of raw of the same concentration in the same liquids. The former sols are now being studied more fully. It may also be mentioned that crystals obtained from some of the sols apparently represent vulcanized rubber in a crystalline condition. They are being made the subject of closer investigation.

elastic properties are essentially similar in kind to those of raw rubber. The following observations made by Whitby and McNally²⁸ will serve to indicate the nature of the elastic phenomena displayed by autopolymerized styrene and to show their general similarity to those of raw rubber.

(a) Both polystyrene and rubber have an "elasticity temperature" below which the material becomes inextensible or "frozen." For rubber, however, this temperature is somewhat below ordinary room temperature.

(b) Polystyrene shows the effect known in rubber as "racking"; that is to say, if stretched while above its elasticity temperature and then, while extended, cooled below that temperature, it retains the extension, and, if then heated above its elasticity temperature, retracts. (This phenomenon can readily be observed in raw rubber by stretching a strip of smoked sheet and then, while maintaining the extension, cooling it in a stream of cold water. The rubber will be found to retain its extension if now released, but when warmed slightly, as, for instance, by holding it in the palm of the hand, will undergo retraction. It should be mentioned that the term "racking" is not very clearly defined and has sometimes been used to denote particularly the extension of rubber at elevated temperatures to lengths greatly exceeding those to which it can be extended without rupture at ordinary temperature. In this usage of the term too, polystyrene can be "racked," i.e. at temperatures well above the elasticity temperature it can be extended to lengths much beyond those at which near the elasticity temperature it suffers rupture.)

(c) Like rubber, polystyrene shows elastic after-effect, i.e. on releasing a specimen which has been stretched, a slow retraction follows on the initial rapid retraction. This is shown by the following experiment. A strip 6 cms. long was placed in water at 95°, stretched to a length of 22.5 cms., and then released under no-load. Table II gives the length of the strip at intervals after its release.

TABLE II
Retraction of Polystyrene

Time after release (secs.)	0	1	7	102	310	900
Length (cms.)	22.5	10	9	8	7.5	7

(d) When the material is stretched and then released, the amount of set left after any time is profoundly influenced by the length of time for which the material has been maintained in a stretched condition. In an experiment, strips of polystyrene were stretched 450 per cent at 95°, and, after being held at this extension for various periods of time, were released. The retraction over a period of time was followed. The data in Table III indicate the character of the results obtained.

TABLE III
Influence of Period of Stretching on Retraction of Polystyrene

Time kept at 450% (mins.)	0	5	10	90
Extension immediately after release (%)	50	150	175	300
Extension 200 secs. after release (%)	0	55	75	150

The following experiment carried out with strips of raw rubber (smoked sheet) shows that the set in rubber is similarly influenced by the length of the period of time for which the material is maintained in a stretched condition. Strips were held at an elongation of 200 per cent at 48° for various periods of time, and, after being released, they were allowed to stand at room temperature for ten days, when no further retraction could be observed. The set remaining is tabulated as E_1 . The strips were then heated for one hour at 100°, and, after they had been allowed to cool to room temperature, the set (E_2) was again measured. The results are given in Table IV.

TABLE IV

Set in Raw Rubber after Extension for Various Times		
Time kept at 200% at 48° (mins.)	Set at room temperature (%) E_1	Set at 100° (%) E_2
5	22.5	17.5
10	27.5	17.5
15	37.5	20
30	40	27.5
60	52.5	35
120	62.5	42.5
240	67.5	47.5

(e) The higher the temperature at which either rubber or polystyrene is stretched to a given extent, the greater is the set remaining on release. This was observed qualitatively for polystyrene. For raw rubber more exact data were secured. Strips of smoked sheet were held at different temperatures for 30 minutes at an extension of 200 per cent, and were then allowed to retract at room temperature for 10 days (set: E_1) and subsequently were heated to 100° and the set (E_2) again measured. Table V gives the results.

TABLE V

Influence of Temperature of Stretching on Set in Raw Rubber		
Temperature during extension	Set at room temperature (%) E_1	Set at 100° (%) E_2
20°	17.5	15
25	22.5	15
35	30	20
40	47.5	22.5
60	55	45
70	70	55
83	90	75

(f) Both with polystyrene and with rubber, the greater the length to which a specimen is extended, the greater is the set present under given conditions. This was determined only qualitatively for polystyrene. For rubber (smoked sheet) the following more exact data were secured.

TABLE VI

Effect of Original Elongation on Set in Raw Rubber

Elongation maintained for 30 minutes at 48° (%)	Set after 10 days at room temperature (%) E_1	Set at 100° (%) E_2
50	7.5	7.5
100	20	17.5
150	27.5	20
200	40	27.5
250	55	30
350	125	52.5
400	142.5	—

(g) The following experiment was carried out on a strip of polystyrene of cross section 2.05 sq. mm. A constant load of 75 grms. was maintained throughout; the temperature was first raised at a definite rate; then lowered; again raised, and so on. The elongation at the end of each period of heating and cooling and at certain intermediate points is shown in the following Table.

TABLE VII

Heating and Cooling Polystyrene under Fixed Load

Temperature Rising	Falling	Elongation (%)
1. 55°		0
65		10.8
75		35.2
80		457
	1 a. 70°	473
	64	481
	40	481
2. 67		481
70		495
79		523
	2 a. 74	528
	70	528
3. 79		560
	3 a. 25	560
4. 79		590

These results indicate a behavior analogous to that of rubber in two respects. (a) During the first period of cooling, it will be noticed, "creep" takes place until the temperature has fallen below the elasticity temperature. (b) During the periods, 1, 2, 3, 4, the final extension increases in each succeeding period, but the difference between the extension at the end of the first and of the second period is greater than the difference between that at the end of any other two successive periods. This clearly recalls the behavior of rubber when sub-

jected to successive cycles of extension and retraction. With rubber, the difference between the first and the second cycles is greater than that between any other two successive cycles (see Whitby, *Plantation Rubber and the Testing of Rubber*, 1920, Chap. XVIII).

(h) It has been shown by Rosbaud and Schmid (*Z. tech. Physik*, **9**, 28 (1928)) that even extremely small loads will cause raw rubber to extend and will even rupture it if allowed to act for sufficiently long periods. A similar behaviour was observed with polystyrene. For example, while a load of 45 grms. applied at 95° to a strip 2.13 sq. mm. in cross section caused relatively rapid stretching and produced an elongation of 122 per cent in 5 seconds, even a load of 5 grms. would produce the same elongation if allowed to act for 225 seconds.

(i) Sols of autopolymerised styrene show many similarities to sols of rubber. At the same temperature and in the same solvent sols of rubber and of polystyrene have similar viscosities. The nature of the solvent affects the viscosity of sols of the two materials in, broadly, a similar way. The lowest viscosities in both cases were observed in the same solvent, viz. acetal.

Poly-vinyl acetate has also been shown to possess elastic properties similar to those of polystyrene.²⁹

The high degree of heterogeneity which has been shown to be characteristic of high polymers in general³⁰ has also been found to exist in elastic polymers. Data showing the heterogeneity of poly-vinyl acetate have already been put on record.²⁹ The following data show that polystyrene is heterogeneous. A sample of polymer prepared by heating styrene for four days at 245° was separated by fractional precipitation with alcohol from solution in benzene into six fractions. The fractions had molecular weights as follows:³¹

TABLE VIII

Fractionation of Thermo-Polymerized Styrene

Fraction No.	1	2	3	4	5	6
Mol. wt.	4405	2914	2632	1979	1274	977

The heterogeneity of autopolymerized styrene was demonstrated by determining the viscosity of sols prepared from fractions secured by means of fractional precipitation and by means of fractional solution.

A sample of synthetic methyl caoutchouc, prepared by the thermopolymerization of 2:3-dimethylbutadiene-1:3, was found to be resolvable into fractions having different properties, as the following data will show:

TABLE IX

Fractionation of Methyl Caoutchouc

Fraction No.	1	2	3
Time of flow of sol (0.854 gm. in 20 ccs. benzene) (secs.)	55.4	67.8	81.3

Direct experimental evidence has also been secured of the fact that natural rubber shows a degree of heterogeneity similar to that of the artificial elastic colloids mentioned above. The diffusion of swollen rubber into the swelling agent was carried out fractionally, fractions being poured off and fresh liquid added from time to time. The sols thus obtained were found, after being adjusted to the same caoutchouc content, to differ widely in viscosity. Diffused rubber was also separated by fractional precipitation into fractions having different properties. The data obtained in this connection will be published elsewhere. They afford conclusive evidence that raw rubber considered as a whole and also diffusion-caoutchouc ("sol" rubber) are far from homogeneous.

In the case of solid polymers obtained from styrene by means of heat, heterogeneity is easily recognizable when the materials are treated with ether, acetone or ethyl oxalate. When the much more highly polymerized product obtained by allowing styrene to undergo autopolymerization is treated with ether or acetone, no solution or apparent swelling takes place, and when treated with diethyl oxalate, only a very slight swelling. When, however, styrene polymerized by heating at 140° or 180° is treated with these liquids, the major part rapidly goes into solution, but there remains a portion which fails to disperse and which on standing settles out as a white powder. This is clearly a fraction of the material more highly polymerized than the rest. If it is separated and treated with benzene (a solvent for highly-polymerized styrene), it readily goes into solution.²⁸

The ease of dispersion of colloidal polymers derived from a given monomer depends on the degree of polymerization. Thus the product obtained by the spontaneous polymerization of styrene disperses far less than the much less highly polymerized product obtained by heating styrene. The fact that rubber, like other elastic colloids, consists of a mixture of polymers covering a rather wide range of molecular sizes is in full accord with observations outlined earlier on its behaviour to a variety of swelling agents. In a homologous series of polymers the extent to which swelling agents are imbibed rises and the ease with which dispersion takes place falls, as the series is ascended. Further, imbibition by and dispersion of a given polymer is different in different liquids. Hence, the proportion of a given sample of raw rubber which will pass readily into the solvent, *i.e.* the proportion of "sol" rubber obtained, differs in different liquids.

As the higher polymers disperse more slowly than the lower, the rate at which a sample of rubber disperses falls as the proportion which has undergone dispersion increases. Even the highest polymers present will, however, if given time, disperse in many solvents, and hence, as already stated, the whole of the rubber disperses in many cases if left to stand for a long time. The protein network present in rubber may possibly retard the dispersion of the caoutchouc somewhat, but can hardly be regarded as the main cause of the slowness with which the higher fractions of rubber disperse. In this connection it may be noted that diffused rubber (obtained by means of petrolic ether), although free from protein, was found after being vulcanized to behave in solvents essentially similarly to ordinary vulcanized rubber. Vulcanized

fatty oils, in which too a protein network is lacking, were observed to disperse more and more slowly, as the period of vulcanization and, as it may be assumed, the degree of polymerization, increased. After the polymerization of a sulphurized oil (containing no free sulphur) had proceeded just to the point at which the material had set solid, the product dispersed readily in benzene, but, as the polymerization was carried further by continued heating, dispersion became slower and slower, and finally a product was obtained, which, like vulcanized rubber, dispersed in benzene only after standing for several years.

A striking case of the difficulty of dispersion due apparently to a high degree of polymerization is presented by poly-methyl acrylate. This material, which was prepared by heating methyl acrylate for half an hour on the water-bath with 0.1 per cent benzoyl peroxide, has a molecular weight so high that it produces no appreciable depression of the freezing point of benzene. It shows greater swelling than any other polymeric product which has been examined by the writer, taking up, *e.g.*, 88.8 times its weight of methyl acetate; yet it failed to disperse in any reasonable time even in good swelling agents.

Not only the ease of dispersion in solvents, but many other properties of organic colloids are influenced by the degree of polymerization. This has been shown to hold for the melting point of a series of polymers such as the polyindenes;³² for the softening point of elastic colloids such as fractions of polystyrene and poly-vinyl acetate; for the ease of cracking on pyrolytic decomposition in the case of polyindenes³³ and polystyrenes, and for the viscosity of sols of a given concentration.²⁹ It also holds for the elastic properties, as may be well illustrated by reference to polystyrene.

As obtained by allowing styrene to undergo spontaneous polymerization at room temperature, polystyrene has a molecular weight of the order of 100,000 or more; it produces an almost inappreciable depression of the freezing point of benzene. The data quoted earlier in regard to the physical properties of polystyrene all refer to such material. As obtained by heating styrene, however, polystyrene has a lower and measurable molecular weight and correspondingly possesses a lower elasticity temperature and poorer elastic properties. A sample prepared by heating styrene for 48 hours under reflux at 140° had a molecular weight, determined cryoscopically, of 1920; and one prepared by heating styrene for 24 hours in a closed vessel at 180°, a molecular weight of 2180. These samples became soft at 40° and readily extensible at 47°, whereas the corresponding figures for autopolymerized styrene were 65° and 75°. While they could be extended readily, their power of retracting was poor and they showed a vast amount of set when the extending stress was removed. At 72° a specimen stretched 800 per cent and then released, retracted to 600 per cent and remained there. A specimen extended to 1400 per cent at 89° and then released, retracted only to 1100 per cent and remained there.²⁸ Compare the data for autopolymerized styrene in Tables II and III. Synthetic rubber also illustrates the influence of the degree of polymerization on the elastic properties. A sample of synthetic rubber made by heating isoprene at 85°, found to have a molecular weight of about 4000—far lower than that of natural rubber—and to give sols of much lower viscosity

than those from natural rubber, had, correspondingly, elastic properties much inferior to those of raw rubber.

Since the properties of elastic polymers derivable from a given monomer are clearly dependent on the degree of polymerization, the properties of a given sample of materials such as those under discussion in the present paper will be determined both by the mean molecular weight of the material and the range of molecular sizes in the mixture of which the material is composed. For the polymeric products obtainable from a given monomer there is probably a certain degree of polymerization which is optimal for the possession of good elastic properties. If the degree of polymerization is too low, the material, while readily deformable, shows poor recovery from deformation, *i.e.*, it shows a large amount of set when allowed to retract after deformation; if the degree of polymerization is too high, the deformability is small and the material is tough, or, in the highest states of polymerization, brittle or "short." In a certain intermediate state of polymerization, the material is both deformable and retractable.

A consideration of data such as those given to illustrate the elastic behaviour of polystyrene and raw rubber shows that the materials are not perfectly elastic; that on allowing them to recover after deformation, recovery is not immediate and complete, but that there is a certain amount of set. The amount of set is dependent not only on the degree of polymerization, but is also greatly influenced by other factors. The longer the period of time for which the material is kept stretched the higher the temperature at which it is stretched and the greater the extent to which it is stretched, the greater is the set.

Further there are three kinds of set, *viz.* (a) that which disappears in time at ordinary temperature, (b) that which remains permanently at ordinary temperature but disappears on raising the temperature ($E_1 - E_2$), (c) that which remains permanently even on raising the temperature (E_2). Set of the first and of the last types, *i.e.* sub-permanent and permanent set, are due chiefly to the lower polymers present. Set of the second kind, which is permanent at ordinary temperature, but sub-permanent at an elevated temperature, may for convenience be referred to as apparent set and is of special interest. It is due to the fact that, although retractive forces are still present in the material, they become apparent only when the internal viscosity of the material is reduced by increasing the temperature.

What has just been referred to as the viscosity might perhaps more correctly be termed the "elastic yield value." The potential retractive forces in the material are unable to effect refraction because the yield value is too high, unless the temperature is raised. As the unqualified term "yield value" has been assigned to the deformation and flow of plastic materials, it would seem desirable in the case of elastic materials to refer to "elastic" yield value. This yield value is significant in connection with other phenomena presented by elastic colloids as well as in connection with apparent set under that aspect of it just discussed. If a strip of polystyrene is stretched and then, while stretched, cooled below its elasticity temperature, it sets hard and retains its

extension indefinitely at room temperature. The material now presents an extreme case of apparent set. If it is warmed to above its elasticity temperature (the temperature at which the elastic yield value is less than the ultimate breaking stress), the elastic yield value becomes reduced sufficiently to permit the potential retractive forces to become effective, and consequently the material retracts. Polystyrene can also be rendered deformable at ordinary temperature if the elastic yield value is reduced by causing it to imbibe a swelling agent. Another phenomenon which is probably due to reduction in the elastic yield value as a result of increase of temperature is the retraction which follows the application of warmth to a strip of rubber under load. This phenomenon is one aspect of what is known as the Joule Effect.³⁴ Similarly the removal of "grain" from milled rubber by means of a hot table depends on the reduction of the elastic yield value. The same effect, viz. the release of internal strains in calendared rubber sheet, may be brought about, as Weigand showed,³⁵ by applying a swelling agent.

The effect of swelling agents in reducing the elastic yield value, and thus rendering elastic colloids which are not normally so, is not confined to the cases, such as polystyrene and poly-vinyl acetate, which have already been mentioned. It is also observable with hydrophilic colloids such as the proteins, silk fibroin and wool keratin. Von Weimarn³⁶ records that when silk fibroin is dissolved in certain concentrated aqueous salt solutions and then precipitated by the addition of alcohol, it shows elastic properties when the degree of hydration is suitable. "As the degree of dehydration increases," he says, "we may witness the realisation of every possible transition stage marking the gradual conversion from the condition of an extremely viscous syrup through a gluey mass to that of an elastic caoutchouc-like jelly (in which condition an *elastic* extension to twice or even four times the former length is possible) and thence to that of masses possessing the consistency of an animal tendon, till it finally reaches the state of an altogether solid mass, so solid that when in thin sheets it may be broken."

Similarly, the elastic properties of wool are greatly influenced by the extent to which the protein is swollen by moisture in the fibre.³⁷ If a wool fibre containing the normal water-content is stretched and then, while stretched, is dried, it will be found to retain its extension on release, but will then re-tract if again allowed to absorb moisture, i.e. if the elastic yield value of the protein is reduced by allowing it to swell somewhat. Advantage is taken of this phenomenon in the treatment of wool known as "crabbing."

Although wool is much less extensible than rubber, there is broadly an analogy, not only in this respect, but also in other respects between the elastic behaviour of wool and of raw rubber. In both cases time plays an important part in the result of any series of mechanical manipulations through which the material may be made to pass. Strictly speaking, there is no such thing in either case as a stress-strain diagram, but only a stress-strain-time diagram. This is perhaps more obvious with wool than with rubber. On loading a wool fibre, there occurs first a rapid extension, which is reversible, and this is

followed by a further, slow extension, which is only partly reversible and is greatly influenced by the length of time over which the load acts, owing to plastic flow of material.

The ultimate explanation of the fact of elasticity in elastic organic colloids cannot yet be given with any sufficient degree of definiteness. It seems desirable to bear clearly in mind that what distinguishes elastic materials from other deformable materials, such as plastic solids or viscous liquids, is, not their ability to undergo deformation, but their power to recover from deformation. The important feature which calls for explanation is not their deformability but their retractability. It has recently been suggested that the explanation of the elasticity of rubber is to be found in the shape of the molecules themselves. The molecules are regarded as helical in shape. It is supposed that on stretching rubber the spirals straighten out and that "the force which causes the extended chains to coil again into spirals when the stress is released is the residual valencies of the double bonds."³⁸ On this view the retraction is due to the attraction of the double bonds in the caoutchouc molecule for each other. But the molecules of many other elastic colloids are saturated or substantially so. Polymers of styrene, vinyl acetate and methyl acrylate, for example, are capable of retracting after extension despite the absence of double bonds. In support of their view that the retractibility of rubber is due to its double bonds Fikentscher and Mark quote the fact that if the double bonds are saturated with hydrogen chloride the product is inelastic. It may, however, be pointed out that caoutchouc hydrochloride is only apparently inelastic, since, although inelastic at ordinary temperature, it shows elastic properties when warmed.²⁷

Further, according to the view of Fikentscher and Mark, mere unfolding of the spiral molecules will permit of rubber being stretched sixfold. Only beyond that extension is the lattice actually stretched, and, they imply, will permanent set appear. The data given on pages 208-209 of the present paper show, however, that permanent set can be produced in rubber at much lower extensions than 600 per cent.

It may well be supposed that the forces which bring about the retraction of elastic colloids after deformation are essentially the same as those which produce cohesion in the unstrained material. Elastic solids stand between brittle solids on the one hand and liquids on the other in regard to the strength of the cohesional forces involved. In a brittle solid cohesion is such that, on applying stress, rupture takes place before change of shape. In a liquid, change of shape occurs with great readiness and the cohesional forces are insufficient to restore the shape. In an elastic solid, subjected to stresses below the breaking stress, the cohesional forces are insufficient to prevent change of shape but sufficient to restore the shape when the stress is removed.

In imperfectly elastic materials, such as are under discussion in the present paper, the phenomena are complicated by the fact that the materials are mixtures of polymers which differ in regard to the strength of the cohesional forces associated with them. It seems probable that the forces involved in the elastic behaviour of these materials are not only, and perhaps not chiefly,

the primary valencies or lattice forces, but secondary forces such as the force of association responsible for the abnormal viscosity of all but the most dilute sols of these colloids.

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STUDIES IN CHRONAXIE*

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The study of the phenomena of excitability in tissues has long been a favorite field of research for physiologists. The interest of chemists in this field is limited to the extent to which drugs will bring about alterations in the excitability and the interpretation of the phenomena, so far as possible, in terms of chemistry.

Fredericq¹ defines the excitability of a tissue as "the property it possesses of answering by a change in shape, state, or position to a modification occurring in the surrounding medium. That modification of the medium we call the stimulus. A stimulus may be either of a mechanical, thermal, luminous, or electrical nature. The stimulus evokes in the tissue an excitation, and this excitation, if transmitted to the active organ, becomes perceptible either by a muscular contraction, or a glandular secretion or by a change in the electrical state of the nerves, muscles, or glands. The real nature of the excitation, of the modification produced by the stimulus in the excitable tissue, we do not know. It may be imagined, according to the work of Nernst, to be an unequal distribution of ions at the boundaries of distinct colloidal phases: a hypothetical view that lacks, however, definite experimental confirmation."

The studies of these phenomena have been primarily of the relation of the stimulus to the modification produced in the tissue. The reason for this is that the stimuli, mentioned above, are purely physical agents and are consequently more readily investigated than the more complex biological phenomena. The stimulus usually employed is an electrical current, because of the ease in which it can be varied. In the early work in this field it was found that there was some minimum value of the stimulus below which no effect was produced. Various degrees of importance were attached to these minimum or threshold values.

Du Bois-Reymond² found that the electrical stimulus was effective only when the circuit was opened or closed; no visible change occurred during the passage of the current. If the intensity of the current was rapidly changed, the tissue would respond; but if the intensity was changed slowly there was no response. Du Bois-Reymond was unable to correlate any change in the threshold value with the time of application. That is, the threshold value was the same whether the stimulus was applied for one second or one hundredth second.

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** National Research Fellow.

¹ *Physiol. Rev.*, 8, 501 (1928).

² "Untersuchungen über thierische Electricität" (1848).

While such material may be quite interesting to physiologists there is little chemistry concerned, so we hasten on to the point at which the interest of chemists begin to crystallize. Lapique¹ and others found that if the stimuli were applied for very short lengths of time there was a drift in the threshold values. He determined a relation between these quantities and defined arbitrarily a purely empirical value as characteristic of the excitability. This empirical value, the chronaxie, is easy to determine and allows one to compare the excitability of one tissue with that of another, which was entirely out of the question in the older method. Moreover, by means of this concept some very perplexing properties of drug action have become quite simple and clear.

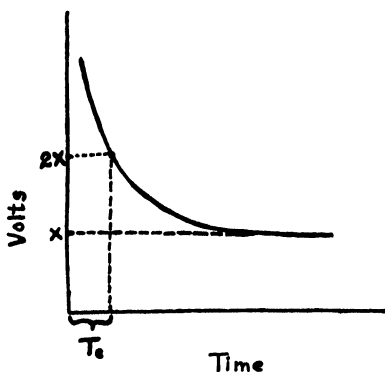


FIG. 1

Let us define chronaxie. The relation between the time of application of the stimulus and the intensity of the stimulus is given graphically in Fig. 1. When the time of duration of the stimulus is very long the minimum effective voltage is x , this value is called the rheobase. If the rheobase is doubled, that is x changed to $2x$, and the shortest time determined at which stimulation will occur it will be found to be T_c , shown on the time axis, this value is called the chronaxie.

The actual chronaxie values vary over wide ranges for different tissues but are constant for any one normal tissue. Thus the chronaxie of the gastrocnemius muscle of the frog is 0.0003 second while the chronaxie of the muscles of the frog's stomach, which are less excitable, is 0.10 second. The investigations of Lapique have brought about a better understanding of the conduction of the nerve impulse from a nerve to a muscle. He was able to show that the chronaxie of a nerve and the muscle to which it was connected were the same. This condition is known as the law of isochronism. Certain drugs can alter the chronaxie of the muscle or nerve and when the values differ by a ratio of about two to one the stimulus is no longer effective; naturally such a condition would result in paralysis. The condition in which the two chronaxies are different is sometimes referred to as heterochronism.

The application of this concept to the action of several drugs can be best studied by starting with curare. If a nerve-muscle preparation is treated with curare and the chronaxies of the nerve and muscle are measured separately it will be found that the value for the nerve is unchanged while that of the muscle is increasing progressively. As soon as the value for the muscle becomes about twice as great as that of the nerve the impulses through the nerve are no longer able to stimulate the muscle and paralysis results. Paralysis would also occur if the chronaxie of the muscle had remained con-

¹ "L'Excitabilité en Fonction du Temps" (1926).

stant while that of the nerve became altered more than the two to one ratio. Thus there are four kinds of curarization, which are outlined in the table below. All of these except the second case have been studied.

TABLE I

Chronaxie of nerve	Chronaxie of muscle	Drug
(1) normal	increased	curare, sparteine
(2) increased	normal	?
(3) decreased	normal	strychnine
(4) normal	decreased	veratrine

This theory also suggests a method of counteracting certain cases of this type of paralysis. Thus if strychnine is used alone it behaves as a curarizing drug, veratrine also has this effect. However, if both drugs are applied at the same time the stimulation of the muscle through the nerve is again possible. The reason for this is easy to see, strychnine decreases the nerve chronaxie until it is below the two to one ratio and paralysis results; veratrine, on the other hand, decreases the muscle chronaxie until it is below the two to one ratio so that if both drugs are acting simultaneously the two new values of the chronaxies, while below normal, are equal which is the necessary condition for the transmission of the nerve impulse to the muscle.

This simple and accurate explanation of the effect of these drugs is in striking contrast with the classical explanation. The older view was that the irritability of the nerve and muscle remained unaltered and the curarizing drug attacked some intermediate neuro-muscular substance which caused the paralysis. Needless to say, the evidence for these mysterious intermediate substances was never formidable.

There are many other interesting applications of the concept of chronaxie to problems in the field of biology and medicine. These application are however outside the scope of this paper, for we are interested in the effect of drugs on the chronaxie of nervous tissue and the variations it undergoes. Before discussing the experiments a brief description of the apparatus and method of measuring the chronaxie will be given.

In the first portion of this paper it was pointed out that the pioneer electrophysiologists erred in assuming that there was no relation between the intensity of the stimulus and the time of its duration. This error arose through the fact that for the frog's nerve-muscle preparation, which was mostly used, time intervals of 0.0003 seconds would be necessary before any marked change in the results would occur. Methods for producing and measuring accurately such short intervals of time were not readily available to the early workers. Even at a later date ballistic methods were used; that is two wires placed at various distances were cut by shooting a bullet through them, the time element was varied by changing the distance between the wires. This method was very inconvenient.

The method now in use depends upon the fact that the time of discharge of a condenser is a function of its capacity and the resistance in the circuit.

Thus, when a variable condenser is charged up to a given potential and then discharged through the preparation of constant resistance the duration of the electrical stimulus is a function of the capacity of the condenser. Such an arrangement is very convenient and requires no unusual form of apparatus.

The diagram of the hook-up is shown in Fig. 2.*

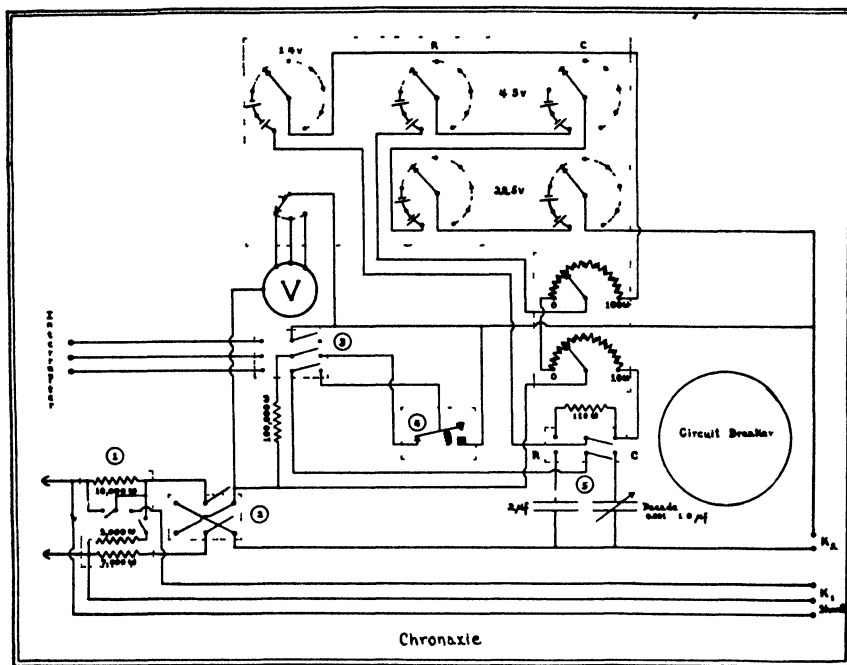


FIG. 2

A source of potential is provided for by ordinary dry cells and radio "B" and "C" batteries; these are arranged behind the panel shown in the upper part of the diagram. In making a determination of the chronaxie two silver wires coated with silver chloride are placed in the physiological salt solution that bathes the preparation; in the experiments in this laboratory this was always the nerve of the muscle-nerve preparation from the frog. These electrodes are connected with the resistance bank (1) and 10,000 ohms resistance placed across them. The switch marked (2) is merely a reversing switch, switch (3) is closed so that the key (4) is in the circuit and switch (5) is thrown to the left side marked "R," for rheobase. The apparatus is now ready for the determination of the rheobase, for the intensity of the stimulus can be varied by altering the voltage, and the time of duration of the applied stimulus will

* The authors are indebted to Dr. Hallowell Davis of Harvard University for the above hook-up and to his kindness for demonstrating to us the technique of measuring the chronaxie of a tissue. We wish to take this opportunity to express our appreciation of his aid. We thank Professor H. S. Liddell of the Department of Physiology for suggesting this work, for placing the apparatus at our disposal, and for his hearty co-operation at all times.

be long because the two micro-farad condenser is the condenser that discharges through the nerve when the key (4) is tapped. The actual determination is accomplished by setting all the switches on the panel at zero and then increasing the potential by turning the smaller value switches up first, each time testing the nerve by tapping the key. When the potential is great enough the muscle contracts. Smaller gradations of the potential can be obtained by altering the resistance of the variable resistance above switch (5). The smallest potential that will cause the first visible twitch of the muscle is the desired rheobase. The actual value can be read off on the voltmeter on the panel.

The determination of the chronaxie, the time required to stimulate the preparation with a stimulus of twice the rheobase, is next carried out. The switch (5) is now thrown on the right hand side marked "C," for chronaxie, this places the variable condenser in circuit. The switches marked "C" on the panel are then altered such that the new potential is just twice that of the rheobase. This new potential of twice the rheobase is now flowing through the preparation and charging the variable condenser. By varying the capacity and testing each time, by tapping the key and discharging the condenser through the nerve, the smallest capacity is sought which will cause the first visible twitch of the muscle. When this is found, the chronaxie is given by the following expression:

$$\text{chronaxie} = \text{capacity} \times \text{resistance} \times 0.37$$

Lapicque evaluated the constant of 0.37 by calibrating against some more accurate method. The need for this constant arises through the characteristic discharge curve of condensers. Bouman¹ and Monnier² have calculated from theoretical considerations the value of this constant and found it to be $3/8$ or 0.375.

Since the resistance is constant, the chronaxie is proportional to capacity and in this study of the variations of the chronaxie the values are given in terms of the capacity directly.

In our study of the coagulation theory of narcosis we were led to the interesting conclusion that there was a very close relation between narcotics and stimulants.³ In low concentrations narcotics behave as stimulants and in high concentrations stimulants behave as narcotics. We believe that both stimulants and narcotics are coagulating agents for the proteins of the nervous system that is involved. After writing our paper, it was pointed out that this state of affairs could be easily investigated by a study of the variations of the chronaxie of the nervous tissue. A rather hasty survey of the field showed that in the majority of the cases the effect of a drug on the chronaxie of a tissue was reported as either an increase or in other cases as a decrease. According to our views, the chronaxie should first decrease, then return to a pseudo-normal state and later go above normal when the proteins of the system

¹ Arch. néerl. Physiol., 12, 416 (1928).

² Soc. Biol. de Paris, 98, 290 (1928).

³ Bancroft and Richter: J. Phys. Chem., 35, 215 (1931).

undergo a sufficient decrease in dispersion. A study of the variations of the chronaxie when the nerve is exposed to a known coagulating agent will give a trustworthy answer to this view.

The first experiment was carried out on the muscle-nerve preparation of the frog. The preparation was placed in a hard-rubber trough and the portion separating the chambers containing the muscle and the nerve was sealed off carefully with vaseline, so that solutions could be added to the nerve chamber without coming in contact with the muscle. Both chambers were then filled with Ringer's solution and the chronaxie of the nerve measured and found to be normal. Mercuric chloride was considered to be a good coagulating agent so one drop of a ten percent solution was added to the chamber containing the nerve. For the first fifteen minutes no change was noted in the chronaxie then suddenly there was a drop of 15-20% below normal. The next measurable value was about 20% above normal. The series of changes took place so rapidly that no more values could be obtained. The initial lag is explained by the slow penetration of the bichloride through the thick sheath of the nerve. The coagulating action of the mercuric chloride is so intense that the changes occur quite rapidly, nevertheless, in several repetitions of the experiment there can be no doubt that the chronaxie first decreases and then, later, increases above the normal value.

A milder coagulating agent was next tried, several drops of a ten percent solution of lead nitrate were added to the nerve chamber. Three or four minutes later the chronaxie was 22% below that of the initial normal value; eight minutes later 46% below normal, this was the minimum value. There was then a steady increase in the chronaxie, ten minutes later the chronaxie was only 16% below normal. A few minutes later the chronaxie was back to the normal level but this was only a pseudo-normal state for the rheobase was much higher than the normal rheobase. The chronaxie continued to increase until the nerve was paralyzed.

It would be of interest to study some organic coagulating agent and also study the changes that take place during recovery. In our study of the colloid chemistry of anesthesia we pointed out that under certain conditions the cell or tissue should pass back over in the reverse order the phenomena it exhibited when undergoing narcosis. The usual muscle-nerve preparation was used and the chronaxie of the nerve was found to be 0.075 micro-farad. Several drops of ethyl alcohol were then added to the nerve chamber and the changes in chronaxie were as follows: 0.068, 0.066, 0.077, 0.083, the preparation was narcotized two or three minutes after the last value was obtained. The solution in the nerve chamber was then removed with a pipette and fresh Ringer solution added; after allowing the solution to remain in the cell for a few minutes it was again replaced with fresh Ringer's and the recovery of the preparation awaited. The time of recovery naturally depends upon the reversibility of the coagulation produced, this in turn is a function of the concentration of the narcotic and the time exposure. For the experiment recorded here fifteen minutes passed before there were any indications of recovery; at the end of this period there were faint signs of twitchings with a potential of thirty volts.

The preparation was again washed with fresh Ringer's solution and the first value obtained was 0.087. Within the next ten minutes the chronaxie had dropped progressively down to 0.060 which was the minimum; from this time on there was a steady increase until the normal value was reached, the over-all time being about forty-five minutes. Throughout the experiment the nerve was washed frequently with fresh Ringer's solution. Thus there can be no doubt that the phenomena of recovery proceed in the reverse order to those of narcosis.

This phenomena of a decrease in chronaxie followed by an increase is not confined to the salts of the heavy metals and alcohol. Anesthetics such as ether do the same thing. In fact this property is so characteristic that in at least one case it has been proposed as a method of standardization for local anesthetics.

Chardot and Regnier¹ have shown that for cocaine hydrochloride solutions the final value of the diminution is a function of the concentration. They note that the phenomenon is reversible and that during the narcotization there is an elevation of the rheobase. The values obtained by them for the percentage depression of chronaxie with different concentrations of cocaine hydrochloride solutions, all at a pH of 6.8 are given in Table II.

TABLE II

% concentration	% depression	% concentration	% depression
0.05	63.	0.010	54.
0.03	62.	0.0075	43.
0.015	55.	0.0050	27.

Another interesting substance, urea, was found also to behave in the typical manner of first lowering then elevating the chronaxie. On washing the nerve with fresh Ringer solution the recovery was also typical in that the nerve passed through the stage of increased irritability (decreased chronaxie) before reaching the normal level. The decrease in chronaxie amounted to approximately 40% of the initial value.

The above cases are not isolated observations, other workers have noted similar changes in the chronaxie of a tissue when exposed to drugs. Thus Lapique² noted that nicotine first decreases then later increases the chronaxie of the gastrocnemius muscle of the frog. S. Weisz³ mentions several other cases. He found this effect for salts of mercury, arsenic, chromium, and manganese and also for carbon disulfide, ammonia, phosphoric acid, methyl alcohol benzene, aniline, and phenol. It is probable that the ammonia was converted into urea and was acting as such; this does not change the situation however, for we have shown that urea also behaves in the typical manner.

Before leaving this subject there is another interesting agent that is worth mentioning. The effect of X-rays on chronaxie have been investigated and

¹ Soc. biol. de Paris, 78, (2) 1247 (1926).

² Soc. biol. de Paris, 73, (1) 654 (1921).

³ Deut. med. Wochenschr., 55, 782 (1929).

the results are quite interesting.¹ Wels found that X-rays can behave as coagulating agents for certain isolated proteins.² He also noted the changes in viscosity, first decreasing then increasing above the normal value, that are characteristic of certain protein sols when undergoing coagulation. Wels was unable to explain the initial decrease in viscosity, so thought that the first measurements might be in error.

X-rays are also able to coagulate the proteins of tissues. In connection with this Heilbrunn³ says: "Williams was able to show very definite effects of X-ray treatment on the protoplasm of the stalk cells of *Saxifraga umbrosa*. Short exposures caused an increase in the rate of protoplasmic streaming, and also an increase in the Brownian movement of the small particles of the protoplasm. Longer exposures caused a diminution of the rate of streaming, and finally complete stoppage. Similar results were also recorded in an early paper of Lopriore. But this worker studied only the rate of streaming and not the Brownian movement. He found that a half hour treatment with X-rays caused an increase in the rate of streaming in the protoplasm of the leaves of *Vallisneria spiralis*. Treatment for an hour caused the protoplasm to become yellow, granular, and coarsely vacuolated. Seckt also found that short exposures to X-rays increase the rate of protoplasmic streaming in various plant cells and that longer exposures slow it. From all these results, it seems probable that X-rays first produce liquefaction, then coagulation of the protoplasm." The changes in viscosity noted above are typical of coagulating protoplasm.⁴ The effect of X-rays is interesting in that they behave as coagulating agents but neither add nor subtract anything in the tissue.

From the previous discussion of the effects of coagulating agents or narcotics on the variations of the chronaxie, it would be expected that X-rays on short exposure would cause also an increase in the excitability, as a matter of fact this was found to be the case. The nerve-muscle preparation was exposed to X-rays (ten milliamperes at a distance of twelve inches) for short periods of time, the maximum time being thirty seconds. At first there was no change in the chronaxie, this was due to the fact that several minutes lapsed before the measurements were made. If the measurements were started immediately after the exposure, the chronaxie was 20-25% below normal and rapidly returns to the normal level, the time of recovery being about three minutes.

Thus from every angle the evidence is accumulating that any agent which causes a decrease in the dispersion of the nerve colloids will reproduce the phenomena of narcosis, which lends considerable credence to the colloid theory of narcosis.

Aside from the ability of this theory to account satisfactorily for the phenomena of narcosis, it is interesting in that it suggests the close relationship between stimulants and narcotics. Thus, as du Bois-Reymond concluded that the agent which gives rise to the excitation of the nerve is not

¹ Wilber: Unpublished Work.

² Archiv ges. Physiol., 199, 226 (1923).

³ Heilbrunn: Colloid Chemistry of Protoplasm, p. 138.

⁴ Bancroft and Richter: J. Phys. Chem., 35, 215 (1931).

the electrical current alone, but that it is due to the variations of the intensity of the current, one could best conclude that the phenomena of irritability are associated with the changes in dispersion of the colloids. For after all, the electrical current brings about an alteration of the dispersion of the colloids of the protoplasm. This was discovered by the physiologists themselves.¹ Heilbrunn,² after summarizing the literature on this subject, concludes that "for both plant and animal cells it seems certain that the passage of an electrical current very quickly produces a reversible gelation or coagulation of the protoplasm." Thus in regions of marked coagulation or peptization the phenomena of excitability are practically absent because it is very difficult to effect a change in dispersion. On the other hand, in a system that is undergoing a decrease in dispersion, as in narcosis, the decreasing stability of the system manifests itself in the phenomena of increased irritability.

Stimulants may be regarded as agents that initiate the change in dispersion of the colloids. According to the principles of thermodynamics, any process tends to occur if it is accompanied by a decrease in the free energy. The free energy of colloidal solutions is associated with the interfacial surface tension; so according to this rule colloidal systems have a tendency to coagulate, for by this process the active surface is diminished. It is not surprising then, to find that the change in dispersion brought about by drugs is usually associated with a decrease in dispersion or coagulation of the colloids of the biological system.

There are many interesting phenomena associated with the increasing excitability that is produced by coagulating agents. In another paper we have advanced the thesis that the increased excitability, if localized in certain tissues, may be associated with some of the phenomena of insanity.³ One can see that, if the central nervous system was either directly or indirectly exposed continuously to coagulating agents, the tissue would be first stimulated then and later paralyzed, if the concentration of the coagulating agent were sufficiently great. The effects of the stimulation may easily be the bizarre mental reactions that are found in insanities caused by coagulating agents. Since we have already shown how the chronaxie of a nerve varies when exposed to lead salts, the following description, from Osler's "Modern Medicine," concerning the mental and nervous symptoms of lead poisoning are interesting. "There may be hysterical symptoms, 'toxic hysteria,' with hemianesthesias and other stigmata, or other hysterical outbreaks of excitement or convulsions, especially in predisposed young women.

"The most common and striking cerebral symptoms are epileptiform convulsions, mania, delirium, and coma; sometimes a picture more or less closely resembling parietic dementia. The outbreak often comes suddenly; alcoholics are especially liable. Convulsions, or less commonly delirium, usually occur first. The convulsive attacks are epileptiform with clonic and tonic movements; only one may occur, but, as a rule, the attacks are repeated at varying

¹ Bayliss: Proc. Roy. Soc., 91B, 196 (1920).

² "Colloid Chemistry of Protoplasm" (1928).

³ J. Phys. Chem., 35, 1606 (1931).

intervals over days—rarely, even weeks. Delusions of persecution and particularly hallucinations, especially of terrifying character, are common; though they are not confined to such subjects, hallucinations are very frequent in those who are also alcoholic, and with marked tremor the resemblance to delirium tremens may be very striking. . . . Most important of all the nervous symptoms is paralysis; in its usual distribution it is of itself almost distinctive of lead poisoning. When typical it produces so-called ‘wrist-drop’ and ‘toe-drop,’ which is bilateral.”

Weisz¹ studied the effect of heavy metals on the chronaxie and found that there was an initial decrease which passed through a point of inflection and then went above normal. He tried the interesting experiment of giving guinea pigs very small amounts of the salts over a period of several days and measuring the chronaxie in the animal every day. He found that the phase of decreased chronaxie corresponded with the increased irritability of the animals and the increasing chronaxie ran parallel with the paralysis.

The variations of the chronaxie of a tissue caused by a decrease in dispersion of the bio-colloids, present another type of curarization that is very interesting from a theoretical viewpoint. If the chronaxie of the nerve and muscle are not the same within the two to one ratio the nerve impulse will not affect the muscle. The cases of curarization, mentioned above, involved the change, increase or decrease, of either the muscle or nerve separately. From our study it is clear that the chronaxie can be either decreased or increased, depending upon the concentration of the drug; or, if the concentration is high, the time of action. Thus if a single drug brought about these variations of the chronaxies of both the muscle and nerve but at different rates in the two tissues one would have the interesting case of a drug antagonizing itself. If the drug would first decrease and then increase the muscle chronaxie, for example, above twice its normal value while the nerve was only in the phase of decreasing chronaxie, a state of heterochronism would exist and paralysis would result. Now if one bathed the nerve in higher concentrations of the same drug, or allowed a longer time of action, if the concentration was sufficiently great, the chronaxie of the nerve would also pass through a point of inflection and rise above normal; as soon as the nerve chronaxie increased to that of the muscle a state of isochronism would again exist and the paralysis disappear. This interesting case of “autoantagonism” has not been realized to the best of our knowledge, nevertheless the existence of such cases is probable and unless one knew of the variations of the chronaxie the explanation would be perplexing.

In summarizing, the situation is briefly that the physiological phenomena that an isolated tissue exhibits when undergoing narcosis can be accurately followed by measuring the alterations in the chronaxie. The first effect of the narcotic is to increase the excitability of the tissue; the second effect is for the excitability to pass through a maximum value, then decrease below normal. The very interesting thing is that indisputable coagulating agents, such as the

¹ Deut. med. Wochenschr., 55, 782 (1929).

salts of the heavy metals, in so far as they affect the tissue, faithfully reproduce the same phenomena. The correspondence between the behavior of known coagulating agents and narcotics is so close that one is drawn to the conclusion, which has also been reached from independent chemical sources, that coagulation and narcosis go hand in hand.

In presenting the colloid theory of narcosis in which coagulation, or decrease in dispersion, of the bio-colloids was the important point, it was a matter of interest to know which type of bio-colloids were undergoing the decrease in dispersion. From the inability of the lipid theory to account for the phenomena of narcosis, except in the most elementary cases, we were led to postulate that if the lipoids had any rôle in narcosis it was of secondary importance and that the proteins of the system were the important colloids to be considered. The physiological evidence, based on a study of the chronaxie, is also in harmony with this view.

In the measurement of the variations of the chronaxie in a changing environment, one has a method of indirect analysis, which, if properly interpreted, will give some idea of the chemical nature of the irritable substance in the nerve. The method that is proposed is based upon the following phenomenon: If the pH of a protein sol is changed from a high value to that of a low value the degree of dispersion of the sol is greatly affected. There is, in general, a small range in the pH scale in which the degree of dispersion is much less than that at any other value, this region being termed the iso-electric point; on either side of the iso-electric point the dispersion becomes greater. For proteins the iso-electric points are quite characteristic, but for lipoids the case is different. If iso-electric points exist at all for lipoids, they are not of sufficient importance to be recorded in the literature and are certainly not characteristic.

From the discussion above of the variation of the chronaxie of a tissue whose colloids are undergoing a decrease in dispersion, we would conclude that in a medium of varying pH the final chronaxies would be at a maximum at the iso-electric point, or that the chronaxie would pass through a maximum at a pH which would be within a reasonable range of that of other proteins whose iso-electric point are known. On the basis of the lipid theory nothing but general confusion can be predicted.

The above experiment of varying the pH of the medium surrounding the nerve and determining the chronaxie as a function of the hydrogen ion concentration was carried out. A series of buffer solutions of disodium phosphate and citric acid whose range varied from a pH of 2.2 to 8 were prepared. The muscle-nerve preparation was placed in the hard rubber chamber and the partition separating the muscle from the nerve was well sealed off with vaseline so that the buffers could be added to the nerve chamber without coming in contact with the muscle. The alkaline buffers were added first. After the addition of each buffer three or more determinations of the chronaxie were made. The buffer was then removed from the chamber and the nerve washed with fresh Ringer solution, then the next buffer was added. The results of the experiments are summarized in Table III.

TABLE III

pH	Average value of the chronaxie	pH	Average value of the chronaxie
2.2	0.057	5.0	0.113
3.0	0.063	6.0	0.073
4.0	0.089	7.0	0.067

There can be little doubt about the chronaxie passing through a well-defined maximum. If the results are plotted graphically, the maximum is found to be at approximately a pH of 4.8. That is, the iso-electric point of the proteins that are responsible for the excitability is at pH 4.8. This value of the iso-electric point is within the range that is commonly found for many proteins. The following values taken from Lloyd, "Chemistry of the Proteins" are interesting in this connection, Table IV.

TABLE IV

Protein	pH	Protein	pH
Cryst. egg albumin	4.8	Collagen	4.8
Serum albumin	4.7	Serum globulin	5.5
Gelatine	4.7-5.5	Myogen (frog)	6.0

Lapicque and Larrier¹ in an analogous manner have studied the variations of the chronaxie of the muscle at different hydrogen ion concentrations. They found that in the neighborhood of pH 4 the chronaxie reached a maximum. They also found the same value for the heart muscle of the frog. The iso-electric points of the irritable substances of the muscle and nerve are so close together that one wonders whether the same material is not responsible for the excitability of both the muscle and nerve. The iso-electric point of the proteins of the muscle as measured by the swelling have been found to be at a pH of 4.8.² Another interesting feature of the data of Lapicque and Larrier, which they fail to emphasize, is that they found a second maximum in the chronaxie at a pH of 6. A protein can be extracted from muscle, which is commonly called myogen; the myogen from frog's muscle has an iso-electric point at a pH of 6.0. Thus there appear to be two irritable proteins in frog muscle. It is also possible that with smaller changes in pH more than one point of inflection may be found for the nerve.

An experiment was carried out using substances other than hydrogen and hydroxyl ions as the coagulating and peptizing agents. While the experiment was not successful it is of sufficient interest to be included within this report. A dilute solution of alcohol was placed in the medium surrounding the nerve and the chronaxie followed until the tissue was narcotized. The alcohol solution was then removed and the nerve bathed in a solution of sodium thiocyanate; as this substance is an excellent peptizing agent it was

¹ Soc. biol. de Paris, 78, (2) 450 (1926).

² Archivio de Scienze Biol., 5, (1924).

hoped that it would reeptide the coagulated proteins of the nerve. In the several experiments carried out with the bromides, iodides, and thiocyanates no acceleration in the rate of recovery was noted. A possible explanation of this result is that the ions do not readily penetrate the thick myelin sheath of the nerve.

This explanation was tested in the following way, a nerve was narcotized by treatment with lead nitrate, the narcotized nerve was then bathed in a dilute solution of sodium iodide. If the iodide ion penetrates into the interior of the nerve it would produce the insoluble lead iodide, and the removal of the lead ions would lead to an improvement in the condition of the nerve, which can be followed by measuring the chronaxie. In the experiment that was tried there was no change in the chronaxie, which apparently means that the iodide did not penetrate into the nerve. In confirmation of this view was the fact that the lead iodide appeared, to the unaided eye, to be deposited only upon the surface of the nerve.

The sluggish penetration of these ions led us to assume that if any ions would be permeable it would be the hydrogen and hydroxyl ions. The above work on chronaxie and pH confirms this.

In conclusion, one can rest assured that the lipoids play a secondary role relative to the proteins in the cell. There are some proteins which are intimately associated with the phenomena of irritability or narcosis; and when these protein sols undergo a decrease in dispersion the tissue undergoes a typical narcosis.

Summary

1. A brief review of the concept of chronaxie and its applications to the problems of drug action is given.
2. In narcosis, the tissue first becomes more excitable than normal, then gradually becomes less excitable.
3. The measurements of the chronaxie of a tissue when it is undergoing narcosis reveal that the chronaxie also changes in the characteristic manner of first decreasing and then increasing above normal.
4. When tissues are treated with indisputable coagulating agents such as salts of lead and mercury the changes in chronaxie are identical with those produced by narcotics. This lends credence to the view that narcotics are substances which are capable of causing a decrease in dispersion of the cell colloids.
5. It is a matter of interest to know what type of colloids are undergoing a decrease in dispersion in narcosis. The measurement of the chronaxie with varying pH is a possible method of indirect analysis, in that it will differentiate between lipid and protein substances if either is associated with the phenomena of excitability; the proteins have characteristic iso-electric points whereas the lipoids do not.

6. If protein colloids are responsible for the irritability, then, according to the colloid theory of narcosis, the irritability should be at a minimum at the iso-electric point of the protein.

7. Chronaxie measurements show that the excitability of the frog's nerve is at a minimum at a pH of 4.8. The excitability of the muscle shows two points of inflection. The iso-electric point of pH 4 corresponds roughly to the iso-electric point of the muscle proteins as found by swelling at pH 4.8. The second iso-electric point of pH 6 is identical with that of the muscle protein called myogen.

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THE PHYSICAL CHEMISTRY OF BACTERIAL AGGLOUTINATION AND ITS RELATION TO COLLOIDAL THEORY*

BY STUART MUDD, R. L. NUGENT AND L. T. BULLOCK

Bacteria, in addition to their obvious medical and industrial importance, offer to the colloid chemist for study relatively reproducible suspensions of a great variety of chemical and physical properties. Although much of this rich field for investigation remains little explored, certain aspects, notably those relating to the stability of bacterial suspensions, have received intensive study. The purpose of the present paper is two-fold: first, to attempt a more nearly satisfactory analysis than is at present available of the physical chemical factors determining the stability of bacterial suspensions, and, second, to offer, regarding the general theory of colloidal aggregation, certain suggestions derived from analysis of these special cases.

The aggregation of bacteria under the action of specific substances in the blood of infected man or animal is a special manifestation of the defensive reactions upon which higher animal life depends. The mechanism of this aggregation has received particularly intensive study. We shall try to reach conclusions regarding the general problem of colloidal aggregation through following in outline the history of the study of this special type of aggregation.

The fact that the phenomena of bacteriology and immunity have been described in a special terminology has been a serious obstacle to the much needed collaboration of chemists in these problems. Let us therefore begin by defining our terms. A foreign substance, which may be a component of a bacterium or other cell or a pure foreign protein, is called an *antigen* if when introduced into the blood stream it gives rise to a corresponding "immune" substance called *antibody*. The liquid part of the blood which is extruded from a contracted blood clot is known as the *serum*, and if it contains specific antibodies it is known as an *antiserum*. The antibodies are designated in terms of the phenomena they bring about by interaction with their corresponding antigens. Thus an antigen in solution may be precipitated by its corresponding antibody, which is then called a *precipitin*. If the antigen is a component of a bacterium or other foreign cell, interaction with antibody may lead to aggregation of the foreign cells; this aggregation is known as *agglutination*, and the antibody involved is called an *agglutinin*.

There is good reason to believe that agglutinins and precipitins may be the same substances merely reacting with antigen under different circumstances.¹ Thus the antiserum formed by injection of animals with crystalline egg albumin precipitates the egg albumin from solution. If, however, the antiserum reacts with egg albumin which has first been adsorbed on collodion particles, these particles are agglutinated.² If now these same agglutinated

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particles are allowed to come into contact with white blood cells the particles are taken within the white cells.³ There is thus strong evidence to indicate that the reaction between antibody and antigen is the essential process which leads to various important secondary phenomena among which are included precipitation, agglutination and the ingestion and destruction of the antigen by the defensive cells of the body. A bacterium or other cell which has combined with antibody is said to be *sensitized*.

Agglutination by antibodies was described by Gruber and Durham in 1896 and precipitation by Kraus in 1897. The great importance of the phenomena soon became apparent and led to the attempt to discover the mechanism by which antibodies act. Two opposing schools developed. The line of cleavage was between the points of view of structural organic chemistry and of colloidal chemistry. Ehrlich and the German school built up the elaborate side-chain theory of immunity. Agglutinins according to this theory possessed certain chemical groupings (haptophores) by virtue of which they combined specifically with their corresponding antigens; other chemical groupings or side chains (agglutinophores) were supposed to bring about the agglutination.

In opposition to the highly elaborated structural chemical side-chain theory, Bordet and the French school postulated a relatively simple colloid-chemical mechanism. "Bordet⁴ made the important observation that *agglutination does not occur if both the bacterial suspension and the agglutinating serum are dialyzed free from salts before mixing*; but if, to such mixtures, a small amount of NaCl is added, agglutination and precipitation of the bacteria occur at once (Reference 1, p. 156). This observation brought the phenomenon of bacterial agglutination into close relation with the precipitation of colloids by electrolytes, Bordet comparing it to the precipitation of particles of inorganic matter suspended in the fresh water of rivers that occurs when the fresh water meets the salt water of the ocean. He found that if the agglutinin combined with the bacteria in the absence of the salts, the resulting compound was precipitated by the addition of minute amounts of electrolytes, which alone did not precipitate or agglutinate the bacteria or the serum. This is a general principle applying not only to the agglutination of bacteria, but also of other cells."⁵

Thus in agglutination by antibodies there are two separable stages, the combination of antigen and antibody, and the subsequent aggregation of the antigen-antibody complex. According to the German school both the combination of antigen and antibody and the subsequent agglutination occurred by virtue of special chemical groupings. According to the French school the combination of antigen and antibody was an adsorption and the subsequent agglutination occurred according to the ordinary rules of colloidal aggregation. The gifted supporters of these conflicting theories and their followers vigorously sought experimental verification, and a wealth of data has accumulated. Only within recent years, however, has it become apparent that each view contained a part and only a part of the truth.

Without attempting to discuss in detail the combination of antigen and antibody we may state categorically the now generally accepted conclusion

that this combination depends upon specific chemical constitution and that the specifically reacting groups may constitute only a very small part of the molecules. Recent work has demonstrated indeed that the specificity of combination between antigen and antibody may be determined by the spatial configurations about a single carbon atom. Thus Landsteiner and van der Scheer⁶ have prepared antigens containing the acyl radicals of the levo-, dextro-, and meso-tartaric acids. Injection of these antigens elicited antibodies which combined specifically with the respective levo-, dextro-, and meso-stereoisomers. Avery and Goebel⁷ have similarly prepared antigens by coupling proteins with p-aminophenol β -glucoside and p-aminophenol β -galactoside. Injected into rabbits, antibodies were elicited which reacted specifically with the particular hexoside contained in the injected material. Specificity of combination in this case was determined by the stereochemical configuration of the hexoside, and was independent of the protein to which it was coupled. A detailed discussion of immunological specificity may be found in Wells': "Chemical Aspects of Immunity."¹

What, then, is the mechanism by which the specific chemical combination of antibody with antigen is able to bring about agglutination? We will first summarize the objective alterations in physical chemical properties resulting from combination with antibody. These have been worked out by Bordet, Bechhold, Porges, Coulter, Northrop and De Kruif, Northrop and Freund, Shibley, Mudd and Mudd, Eagle, and Mudd, Lucké, McCutcheon and Strumia. With these data before us, we will discuss earlier experiments and theories, and will finally attempt a more nearly adequate treatment than is at present available.

Various bacteria vary widely in the chemical composition and colloidal properties of their surfaces. Thus virulent pneumococci possess capsules composed largely of certain complex polysaccharides;⁸ in the "rough" variants of pneumococci these carbohydrates are lacking and the surfaces are chiefly protein. Certain bacteria isolated from tuberculosis of birds or of cold-blooded animals behave as though their surfaces were largely lipoid.⁹ In an oil-water interface most bacteria show decided preferential wetting by the aqueous phase; many "acid-fast" bacteria under the same circumstances are wet preferentially by the oil.^{9,10} Certain microorganisms have definite isoelectric points; others retain a negative surface p.d. even down to pH values below 2.0; we have cultures of at least three strains of "intestinal" bacteria which show only a minimal surface p.d. in buffers of any pH studied. Some bacteria readily form stable suspensions; others may be suspended only with difficulty and undergo spontaneous aggregation.

Interaction of these bacteria each with its corresponding specific immune serum masks this great diversity. As the bacteria combine with progressive concentrations of their corresponding immune sera, their surface properties progressively converge toward a new and common set of surface properties.

This convergence for the isoelectric points is shown in Fig. 1. Each bacterium used (except *S. pullorum*) had been isolated from a human patient; the bacterial suspension was allowed to stand overnight in contact with serial

dilutions of the serum of that patient.* Appropriate controls showed that the resulting combinations with antibody were specific. The sensitized bacteria were washed in 0.85% NaCl solution and their isoelectric points were determined in acetate buffer series with the aid of a Northrop-Kunitz microcataphoresis cell.¹² Before sensitization the staphylococcus retained a negative

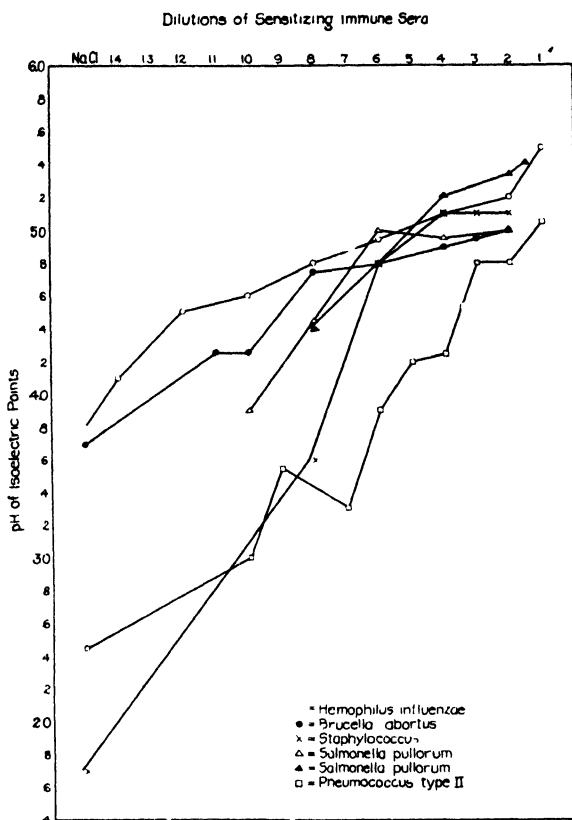


FIG. 1

Convergence of isoelectric points of various bacteria specifically sensitized with human immune sera. Abscissae are the final dilutions of immune sera in 0.85% NaCl solution used to sensitize the bacteria. These are expressed as powers of 2; thus 5 indicates a dilution of 2⁵ or 1 volume of serum diluted to 32 volumes with NaCl solution. Ordinates are the isoelectric points of the sensitized, washed bacteria as determined in a microcataphoresis cell. As the concentration of sensitizing serum increases the diverse isoelectric points shift to maximal values of pH 5.0 to 5.5, thus approximating the isoelectric point of serum globulin.

charge even in N/100 HCl, the pneumococcus was isoelectric between pH 2.0 and 3.0 and *H. influenzae* and *B. abortus* between pH 3.0 and 4.0 and *S. pullorum* had little if any surface p.d. in any buffer used. With progressive sensitization the isoelectric points of all the bacteria shifted progressively until values of pH 5.0 to 5.5 were reached.

* *S. pullorum* was sensitized with the sera of typhoid fever patients, with which *S. pullorum* interacts specifically.¹¹

Concomitant with this convergence of isoelectric points with progressive sensitization, there is a convergence of other surface properties. The surface p.d.'s change to a common value, usually lower but in some cases higher than the unsensitized value.¹³ The wetting properties, which may have been hydrophilic or hydrophobic in the unsensitized state, become those of protein.¹⁴ The cohesiveness of the sensitized bacteria becomes great. Concomitantly also with these changes in surface properties there occurs the agglutination which has been discussed. The extensive data on which these summary statements are based may be found in references 3, 13 and 15, and in earlier works there referred to.

The explanation of this convergence of surface properties, as is no doubt evident, is that the antibodies which combine with the antigens of the various bacteria or other cells form deposits or films, which do not differ greatly one from another, on the antigen surfaces. The properties of these films are similar to those of denatured serum globulin,^{13,14} but not, at least in some cases, identical with them.^{3,16} It is probable that the antibody is itself a modified serum globulin or at least contains serum globulin as a constituent part. The most noteworthy difference of the antibody-globulin from normal serum globulin is the specific combining affinity of the antibody for antigen. The sensitized surface has in addition to the properties already described the property of adsorbing non-specific globulin and albumin (complement) from the serum.¹⁷ The final surface thus built up upon the antigen is thus probably a composite film, composed of antibody-globulin specifically combined with the antigen and more or less adsorbed non-specific serum protein. Specific serum sensitization thus makes many chemically diverse particles essentially similar in their surface properties and markedly increases their tendency to agglutinate. From the point of view of immunity the important fact is that this sensitization process renders the foreign particles susceptible to ingestion (phagocytosis) by the defensive cells of the body; however, ingestion by cells is not within the scope of the present paper. This arrangement, by which many diverse substances are given a common set of properties that can in the main be dealt with successfully in the body, were it the product of human intelligence, would be an extremely ingenious solution of a difficult chemical problem.

With these recent data with regard to the sensitizing antibody film before us let us return to consideration of such earlier experiments and theories as throw light upon the physical chemical factors affecting the stability of suspensions either of unsensitized bacteria, sensitized bacteria or of both. The basic discovery of Bordet that sensitized bacteria are stable in distilled water but are agglutinated by traces of electrolyte was extended by Bechhold in 1904.¹⁸ Bechhold showed that the cation was the important precipitating ion and that its efficiency increased rapidly with valence. The bacteria studied by Bechhold, i.e. typhoid and dysentery bacilli and staphylococci, with respect to precipitation by electrolytes resembled emulsoid colloids; after sensitization by specific serum they behaved more nearly like suspensoids. These general results were confirmed and extended by many investigators.

Tulloch¹⁹ concluded in 1914 that "as unsensitized organisms behave—towards electrolytes as does fresh egg protein, but when sensitized have characters that recall those of denatured egg-white, the process of sensitization is akin to that of denaturation."

Buchanan²⁰ in 1918, devoting his presidential address before the Society of American Bacteriologists to the subject of bacterial agglutination, reviewed the earlier work and proposed his own theory of the stability of bacterial suspensions. Buchanan regarded the negative *charge* as the stabilizing factor and "*surface tension*" as the aggregating factor. "We may regard the similar electric charge as constituting the repulsing agency, the surface tension as the attracting agency. A study of the agglutination phenomenon then resolves itself into a consideration of the means whereby these two forces may be modified, increased or diminished. Agglutination occurs whenever the similar electric charges are decreased to amounts such that they will no longer overcome the pull of surface tension. Or conversely, surface tension may be increased until it overcomes the dispersion effect of the similar charges."*

Herzfeld and Klinger²³ about the same time attributed the stability of bacterial suspensions to the water-solubility ("Hydrophilie") of the surfaces due to adsorption of soluble decomposition products. Electric charge according to Herzfeld and Klinger played only a subordinate part.

The work of Northrop and De Kruif^{24,25} and Northrop and Freund²⁶ marked an important advance in this field. Their studies were of peculiar importance for at least two reasons: firstly, that they substituted measurement of the physical factors involved for speculation about them, and secondly that they laid the foundation upon which the modern conception of the sensitizing antibody-protein surface film has been established.

The *electrokinetic p.d.* was calculated in this work from direct measurements in an improved microcataphoresis cell. Bacteria were dried in thick films upon plane glass surfaces; these were immersed in aqueous solutions of various salt contents, were allowed to cohere, and the force required to separate them was determined by a du Nöuy tensiometer. The relative *cohesion* between bacteria in various suspending solutions was thus measured.

Bacteria in electrolyte solutions of .001 M concentration or less were found to be stable if the electrokinetic p.d. exceeded the critical value of ± 15 millivolts. The critical potential for unsensitized red blood cells was about ± 6 millivolts, for sensitized red blood cells about ± 12 millivolts. Within these critical zones both types of cell aggregated when the electrolyte content of the medium was low. At higher concentrations of electrolytes, .01 M to 0.1 M, aggregation sometimes occurred within the critical potential zones, sometimes did not. In salt concentrations exceeding 0.1 M aggregation rarely occurred even though the electrokinetic p.d. was reduced to zero. Bacteria

* Surface p.d. or electrokinetic p.d. and surface "charge" are both manifestations of the existence of an electrical double layer at the particle-dispersion medium interface. The direct stabilizing action of the electrical double layer is believed to be due to the mutual repulsion of similarly charged surfaces.²¹ The electrokinetic potential difference is proportional to the surface charge²² and since it is the measurable factor, it will hereinafter be frequently referred to as the stabilizing influence.

sensitized with immune serum were regularly agglutinated when the p.d. was brought within the critical limits regardless of salt concentration.

Measurement of cohesive force between the unsensitized bacteria showed progressive reduction with increasing electrolyte concentration. Over the range of concentration of 10^{-4} N to N this reduction in cohesive force between unsensitized bacteria amounted to approximately 50%, according to the figures of Northrop and De Kruif. The cohesion of bacteria sensitized with immune serum was high and was not affected by salt concentration.

These results were summarized by Northrop and De Kruif in part as follows: "Electrolytes in low concentration, (0.01 N), affect primarily the potential, and in high concentration decrease the cohesive force.

"As long as the cohesive force is not affected, agglutination occurs whenever the potential is reduced below about 15 millivolts.

"When the cohesive force is decreased the critical potential is also decreased, and in concentrated salt solution no agglutination occurs even though there is no measurable potential.

"The addition of immune serum prevents the salt from decreasing the cohesive force between the organisms, and agglutination therefore is determined solely by the potential, provided excess immune body is present. Whenever the potential is decreased below 15 millivolts the suspension aggregates."

This work leaves little doubt that the electrokinetic p.d. is a major factor in the stability of bacteria as of other colloidal suspensions. It provides definite quantitative evidence also that cohesion is a factor of major importance. Ordinary bacteriological experience affords sufficient confirmation of the importance of cohesion. For instance young glycerol-agar cultures of a certain acid-fast bacterium, *Mycobacterium chelonci*, (turtle bacillus), form even and stable suspensions with ease when shaken up in salt solution. The same cultures, aged and somewhat dried out, it may be impossible to suspend uniformly, even with the aid of much grinding and shaking. Further confirmation is yielded by the "resuspension" and "interfacial" methods which afford direct, although only semi-quantitative, evidence of the cohesion of bacterial clumps when subjected to shaking and to the dispersing action of an oil-water interface. Hundreds of such observations have shown that sensitized bacteria are much more cohesive than the same bacteria before sensitization.

Since the work of Northrop and de Kruif, Kruyt²⁷ and his associates at the van't Hoff laboratory in Utrecht have in recent years made extensive studies of emulsoid colloids. The hydration of the disperse phase is estimated from careful viscosity measurements, and the p.d. by cataphoresis. These studies have demonstrated two major stabilizing factors for hydrophile systems, *electrokinetic p.d.* and *hydration*. If the disperse phase is dehydrated by alcohol or acetone stabilization depends, as in suspensoid systems, principally upon charge. It is obviously necessary to include this unquestioned influence of hydration in any completed theory of bacterial agglutination.

The studies of dehydration by tannin made by Bungenberg de Jong in Kruyt's laboratory are of especial interest for our purpose. Carbohydrate or protein emulsoids are given by tannin the properties of suspensoids. This action was explained in an early paper by de Jong²⁸ as follows:

"We suppose that here the dehydration is a phenomenon caused by the adsorption of the tannin on the lyophilic particles, by which the residual affinities, which otherwise bind the water of hydration, become partially neutralized by the tannin; the tannin on the other hand does not bind any water, but removes it from the particles. . . .

"Perhaps the theory of Langmuir-Harkins may give us insight into the direct cause of this dehydration. The tannin will be adsorbed in an orientated state. With the colloid carbohydrates the glucose side of the tannin molecule will direct itself towards the surface of the sol particles, i.e. inwardly. The phenol groups of the digallic acid residues will be directed towards the external phase. Now the surface of contact of the sol particles consists of a great number of feebly lyophilic phenol groups. Also, with the adsorption on proteins we are to suppose, that the phenolic groups in tannin adsorbed are outwardly directed, so that there is no more opportunity for important hydrations."

The important thing is that surface films of tannin are bound around protein particles. The tannin surfaces are not hydrophilic. The stabilizing action of hydration is thus lacking and the particles aggregate.

Similar,²⁹ though quantitatively less, dehydration could be brought about by synthetic tannins and by simpler polyphenols. The dehydration of protein sols is relatively greatest at the isoelectric point. The dehydrating power increases rapidly with the number of phenolic groups in the molecule, although simultaneously other influences help to determine the dehydrating power.

Later work^{30,31} afforded evidence of a definite separation of a tannin- or polyphenol-rich liquid phase about the surface of the particle. "The dehydration is attributed to an actual phase boundary between the adsorbed tannin-rich layer and the tannin-poor dispersion medium." However this may be, work in this laboratory has shown evidence of the building up of an adsorption film on particles exposed to increasing concentrations of tannin and then washed. This work will be described in more detail later.

The results obtained by Kruyt, de Jong and others working in Kruyt's laboratory have clearly demonstrated that the question of the state of hydration of surfaces is a factor of major importance and must be dealt with in any general consideration of the agglutination of bacteria. These conceptions have been applied to serology by Reiner and his associates. They have made an important contribution to serology by demonstrating that precipitation, agglutination,³² adsorption of complement³³ and the preliminary steps leading to lysis³² and to ingestion by white blood cells³⁴ could all be brought about by treatment of cells with tannin. Thus the effects of antibodies were imitated in a striking manner by a substance of less complex chemical constitution. These results have been confirmed and extended by Freund³⁵ and by Neufeld and Etinger-Tulczynska.³⁶

On the basis of the similarity of these effects of tannin and antibodies Reiner has advanced a "dehydration" theory of antibody action. Reiner's theoretical treatment of aggregation³² we believe to be unsound for reasons that will be set forth in more detail later. Moreover, whereas Reiner's contribution in bringing the importance of hydration to the attention of serologists is apparent, the justification for singling out hydration in this way from among the several controlling physical factors, is by no means apparent. In fact we find no justification which seems to us adequate, theoretically or empirically, either in Reiner's papers or our own work.

A brief description has now been given of some of the more important general discussions of bacterial agglutination, which have appeared up to the present time. These discussions apply both to unsensitized and to sensitized bacteria. Buchanan's discussion on the basis of charge and surface tension, fails to include a discussion of the importance of hydration of bacterial surfaces. Northrop and De Kruif confirmed the importance of surface charge on the basis of quantitative measurements and demonstrated that a second factor must be taken into account, which they call "cohesive force." Their method of measuring "cohesive force" has been described. They were not inclined to identify it with surface tension.³⁷ It is apparent that they too do not fully discuss the question of surface hydration.

Reiner insists on good grounds that surface hydration must be considered as a factor of major importance in the agglutination of sensitized bacteria. He believes that he has been able to formulate the general question of the stability of bacterial suspensions in terms of the "attraction" of the bacteria which he defines in terms of free surface energies. The views of both Northrop and De Kruif and of Reiner are widely quoted.

It is apparent that no writer on bacterial agglutination to date has properly defined the interrelationship of surface tension, cohesion, hydration, and surface charge, as affecting the stability of suspensions either of unsensitized or of sensitized bacteria. Further, so far as has been ascertained, no writer on pure colloid chemistry has described this interrelationship in such a way that it may be directly applied to the question of bacterial aggregation.

In the remaining portion of this paper, it is therefore proposed first to discuss in so far as necessary, the general question of the factors affecting the stability of suspensions of colloidal particles, and then to discuss the stability of suspensions of unsensitized and of sensitized bacteria on the basis of previous work, the theoretical discussion to be presented here and of experimental observations made in connection with the preparation of the present paper. It is found that certain implications, new even in general colloidal theory, emerge from this process.

The Stability of Dispersions of Colloidal Particles in Water and Aqueous Solutions

The purposes of the present paper do not require an exhaustive treatment of the subject of the aggregation of colloidal particles, but rather a working outline of the subject to use as a basis for the consideration of those phenomena

of bacterial aggregation which are of importance in bacteriology and immunology.

There are two necessary conditions for the existence of a stable dispersion of colloidal particles in an aqueous medium. The first is that the mass and size of the particles be small enough so that they remain suspended for the period under consideration against the force of gravity.³⁸ The second is that some factor must operate to prevent the aggregation of the particles to form larger masses which would no longer remain suspended.³⁹

The mass and specific gravity of bacteria is such that if no appreciable aggregation occurs, the amount of settling which takes place in eighteen hours is sufficiently small to be neglected. For this length of time therefore, bacterial suspensions may be treated as suspensions of colloidal particles. This fact is important, because the agglutination phenomena which are important in bacteriology and immunology ordinarily take place within eighteen hours. The first necessary condition for the existence of a stable suspension of particles is met by suspensions of single bacteria and therefore need not be further discussed here.

Given a dispersion of particles in water whose mass and size are sufficiently small so that settling is slow, three general factors must be considered as determining whether or not appreciable aggregation of the particles takes place within a given period of time.⁴⁰ The first condition requisite to the aggregation of particles is that their Brownian motion must bring them into contact with one another. Therefore the first general factor which must be considered is the rate at which Brownian motion tends to bring this about. The major experimental factors affecting this rate are the concentration, or number of particles per unit volume of the dispersion, the mass and size of the particles, the viscosity and the temperature of the suspension.⁴¹ The study of variation in these factors upon the rate of agglutination of bacteria is of theoretical interest. However the observations of the agglutination of bacteria which are of direct importance in bacteriology and immunology are made under fairly constant conditions, so far as these factors are concerned. These usual conditions are such that the expectation of collision due to Brownian motion in the absence of repelling force is sufficient to cause rapid, complete aggregation of the bacteria if each opportunity for collision results in contact of the particles, and each contact results in cohesion. For these two reasons further discussion of the effect of variation in factors which affect the rate of opportunity for collision due to Brownian motion is unnecessary, from the point of view of the purposes of the present paper. Complete discussion may be found in comprehensive treatises on colloid chemistry.⁴² Northrop has discussed these factors in relation to bacterial agglutination.¹⁵

The second general factor which must be considered as determining whether or not appreciable aggregation of colloidal particles will take place within a given length of time is the probability of contact when opportunity for collision is provided in consequence of Brownian motion. It is clear that such contact must occur unless prevented by some repelling force acting between two particles tending to collide in virtue of their Brownian motion.

If this repelling force is sufficient to overcome the momentum of the particles contact will not occur. There must obviously be a value of any such repelling force at which it just balances the momentum of the particles. This may be called the critical value of the force in any case.

The critical value described is strictly applicable only to a single pair of particles tending to collide under given conditions. In any system of dispersed particles, there is a statistical distribution of velocities due to Brownian motion.⁴³ Therefore a given repelling force may prevent certain collisions and not others in any given case. The critical value of the repelling force for a system of dispersed particles is therefore the value which is just sufficient to prevent a sufficient majority of contacts from taking place when opportunity is offered for collision.

The third general factor is the probability of cohesion after contact has been made. The interface between a dispersed particle and its dispersion medium is the seat of free surface energy equal to the free interfacial energy per unit area multiplied by the surface area of the particle. Contact of two particles results in a decrease in free surface energy* equal to twice the area of contact of the particles, times the interfacial tension, or:

$$\Delta F = 2S\gamma_{AB}$$

where ΔF is the decrease in free surface energy, S is the area of contact of the two particles in contact and γ_{AB} is the free interfacial energy per unit area.

Following Harkins⁴⁴ general treatment of work of cohesion, it is apparent that the work of cohesion between two such particles in contact is measured by the free energy increase necessarily attendant upon their separation under ideal conditions**, or:

$$W_c = 2S\gamma_{AB}$$

where W_c is the "work of cohesion" and the other symbols have the same significance as before.

In order to cause separation after contact has been made, the dispersive forces† must provide a minimum energy equal to W_c . If W_c is greater than

* The free interfacial energy as considered here involves any effects due to the existence of an electrical double layer at the particle-dispersion medium interface. It is a composite result of the interaction of three sets of force fields, those of the particle molecules, those of the dispersion medium molecules and ions and that due to the existence of the aforementioned double-layer. No assumption is necessary here, and none is made as to the equality of the interfacial tension at the micro-particle-dispersion medium interface referred to and the interfacial tension at a macro-interface between the dispersion medium and the material of which the particles are composed.

** The question of the separation of particles under non-ideal conditions will be discussed later in connection with Figs. 4, 5, 6 and 7.

† Exact definition of these dispersive forces is difficult or impossible. It seems, however, that at least three factors may be recognized in a qualitative way:

- (1) The Brownian motion itself.
- (2) Electrostatic repulsion. Hydrophilic colloids which are ionogenic at least owe part of their electrokinetic p.d. to ionization at fixed points on the particle surface. All of these points obviously can not be in contact. It is probable therefore that there is some residual electrostatic repulsion even between particles coherent over a part of their surfaces.
- (3) The tendency of the water molecules to wet hydrophilic substances in the surfaces of the coherent particles may tend to force these coherent surfaces apart.

It is also possible that statistical fluctuations in the internal energy of the molecules of colloidal particles in contact must be taken into account in a complete treatment of dispersive forces. (In this general connection see Burk: J. Phys. Chem., 35, 2446 (1931).)

the energy provided by the dispersive forces the particles will cohere after contact. If it is less the particles will separate again after collision. Obviously here too, as in the case of the repulsive force, there must be a value of W_c which will just balance the dispersing tendency in any case. This may be called the critical value of W_c .

As in the case of the concept of a critical repelling force, so also in the case of the concept of a critical work of cohesion, it is important to bear in mind the statistical distribution of kinetic energies. The Brownian motion impulses tending to separate particles in contact vary in magnitude in a statistical manner. The critical work of cohesion for a system of dispersed particles is therefore such that a sufficient majority of the impulses tending to separate particles in contact fail to do so.

It is apparent from the foregoing considerations that if the repelling force is greater than its critical value, a dispersion will be stable. The same is true if the work of cohesion is less than its critical value.

The question of the variation of the work of cohesion merits particular discussion at this point, remembering that the present discussion applies to dispersions in aqueous media. The work of cohesion has been defined as equal to $2\sigma\gamma_{AB}$. The variation of work of cohesion from case to case is therefore primarily a matter of variation in the free surface energy at the respective particle-dispersion medium interfaces. In general, in accord with Harkins,⁴⁶ the more nearly similar the dispersion medium and the surface material of the particles, the lower the expected interfacial tension. In this connection, it is most important that certain colloidal particles have the property of associating themselves with large quantities of water from their dispersion medium.⁴⁶ It is not necessary at this point to discuss the possible mechanisms involved⁴⁷ in the taking up of the water. It is highly probable that different mechanisms are operative in different cases.⁴⁸ It is however also highly probable that in some of these cases the combination of the particles with water results in a hydrous particle surface, which is much more similar to water in the Harkins sense than the surface of a particle of the same substance in the anhydrous condition. In such cases the interfacial tension of the particles against their dispersion mediums is presumably lowered and along with it the work of cohesion of the particles. It would seem that the lowering of work of cohesion due to the hydration of the surface of dispersed particles must be considered as a potential factor affecting the work of cohesion between them and thus the stability of dispersions of such particles in aqueous media.

The relationship of the foregoing material to the well known experimental facts with regard to the stability of colloidal solutions is fairly obvious. Colloidal particles dispersed in aqueous media, may be conveniently considered in two classes for purposes of discussion, as hydrophobic particles and hydrophilic particles.⁴⁹ The first class have little or no affinity for water and the second a marked affinity.

In the case of dispersions of hydrophobic particles clear-cut experimental evidence⁵⁰ has shown that the necessary condition for their stability is that the electrokinetic potential difference at the surface of the particles exceed a

certain limiting value known as the critical potential.⁵¹ When the electrokinetic potential falls below this value aggregation of the particles takes place. The electrokinetic potential difference between particles and their dispersion medium results from the existence of an electrical double layer at the surface of the particles. The particles are positively or negatively charged with respect to the medium depending, respectively, upon whether the positive or negative side of the electrical double layers is associated with the particles.⁵² It is believed that the repelling action of similarly charged particles is responsible for the stability of suspensions of hydrophobic particles when the electro-kinetic potential exceeds the critical value.⁵³

In terms of the general working theory of suspension stability which has been presented, this type of electrostatic repulsion is the repelling force which if sufficient can prevent the contact of particles when opportunity for their collision is provided in virtue of their Brownian motion, and thus stabilize the dispersion. So far as is known, there is no other type of repelling force to be considered. The critical potential in the case of a single collision may now be defined as that electrokinetic potential difference which is just sufficient to prevent the contact of two particles when Brownian motion offers the opportunity for their collision. The concept of the critical value of a repelling force for a system of dispersed particles has been discussed.

Turning now to the question of the stability of hydrophilic colloidal particles, it is found that quite a different situation prevails. Kruyt and others working in his laboratory⁵⁴ have clearly demonstrated that the condition of hydration of certain hydrophilic colloidal particles must be considered as a stabilizing factor in dispersions of such particles in aqueous media. The first outstanding fact is that certain of the particles studied formed stable dispersions when their repelling force, as measured by their electrokinetic potential, was reduced to zero.⁵⁵ The addition of sufficient alcohol to such isoelectric dispersions caused them to precipitate. The alcohol in such cases is generally considered to act by dehydrating the particles. These facts indicate first that a stabilizing factor is active apart from electrokinetic potential difference, and secondly that this stabilizing factor results from the hydrated condition of the particles. It is apparent that this second stability factor is capable of stabilizing a dispersion of these particles in complete absence of a repelling force. It must therefore act by decreasing the work of cohesion of the particles below the critical value or by raising this critical value. Reasons for this stabilizing action associated with the hydrous condition of the particles then follow directly from the previous discussion. It was pointed out that increased surface hydration should accompany the union of hydrophilic particles with water of the dispersion medium. This increased surface hydration should cause the hydrated particles to have a much lower surface tension against the aqueous dispersion medium than would the same particles in a hypothetically anhydrous condition. Certain hydrous particles might well thus have very low surface tensions against aqueous media which in turn would cause them to have very low works of cohesion, even possibly below the critical value. In this way the hydrous condition of particles in

certain cases could be a stability factor which could result in the stability of a suspension of such particles even when their electrokinetic potential was reduced to zero. Moreover the tendency of the water to wet the hydrophilic surfaces might promote dispersion and therefore necessitate a high critical value of the work of cohesion.

The mechanism outlined above is here offered as the one which is operative in the unquestioned stabilizing influence of the hydrous condition of the particles in hydrophilic suspensions. It is significant that the only dispersions of colloidal particles in aqueous media which are known to be stable in the absence of electrokinetic potential are those in which independent evidence points clearly to the hydrophilic nature of the particles.

It should be pointed out that the concept of variation in the work of cohesion with surface hydration applies to variation in the state of hydration of a particular surface. In passing from one surface to another, as in the deposition of a protective or sensitizing film, no such relationship necessarily exists. Surface A may be less hydrous than surface B and still have a lower work of cohesion. The point is that surface A for example presumably has a lower work of cohesion in a relatively hydrated state than in a relatively dehydrated state. In the sense of this paper, changes in hydration of a particular surface such as may be brought about by the electrolyte content of the medium are considered as modifications of an existing surface rather than the formation of a new surface.

The critical potential as experimentally determined for a system of dispersed particles is the minimum electrokinetic potential compatible with a stable condition of the dispersion under the defining conditions. Suppose that the work of cohesion is sufficiently high so that practically every contact results in permanent coherence of particles. The experimentally determined critical potential will then be such that it is just sufficient to prevent a sufficient majority of contacts when opportunity for collision is offered due to Brownian motion. If the work of cohesion is somewhat lower, that is if an appreciable number of contacts result in redispersion, the repelling force would not have to be quite so large, that is it would not have to prevent as many contacts as before in order to maintain stability. In this second case, the experimentally observed critical potential would be somewhat lower than in the first.

Theoretically therefore, experimentally determined critical potentials should decrease with decrease in work of cohesion in all cases where the work of cohesion is less than sufficient to prevent practically all redispersion. When the work of cohesion is sufficiently small the suspension will be stable even at zero electrokinetic potential. In this connection it is interesting that March, using several assumptions, has calculated that the charge corresponding to the critical potential observed by Powis for stable, pure oil-in-water dispersions is not sufficient alone to account for the stability of the system. The indication therefore is that the work of cohesion of the oil droplets is lowered in consequence of the existence of some form of hydrous interfacial film. (See March: *Koll.-Z.*, **45**, 97 (1928); Heymann: *Koll.-Z.*, **48**, 195 (1929).

Shibley⁵⁶ confirmed the critical potential value of Northrop and De Kruif (± 15 millivolts) with bacteria suspended in NaCl, ZnSO₄, and CeCl₄. In the presence of Na₂HPO₄, however, the same microorganisms had a much higher critical potential (-34.6 millivolts in one experiment). It is possible that the higher critical potential in the presence of Na₂HPO₄ was due to an increase in work of cohesion due to this particular salt.

Aggregation occurs when the electrokinetic potential difference is lower than its critical value in any system of dispersed particles, whose cohesive force is above its critical value. However the *rate* of aggregation within the critical potential zone varies with the residual charge upon the particles, being most rapid at the isoelectric point.⁵⁷ One reason is that the lower the charge the greater the majority of total opportunities for collision which result in contact, with all opportunities for collision resulting in contact at the isoelectric point. Further, it has been suggested that residual charge is a factor aiding the redispersion of particles after contact, at least in some cases. In these cases, the greater the residual charge, the greater the redispersion tendency and presumably the slower the rate of aggregation.

The relationship of stabilizing and sensitizing surface films to the general question of the stability of dispersions of colloidal particles in aqueous media is secondary to the factors which have been discussed. It will be treated in the section of the paper dealing with the stability of suspensions of sensitized bacteria.

As has been mentioned earlier in the paper, Reiner, in connection with his excellent experimental work on the analogous behaviour of bacteria which have been treated with antisera and tannin solutions, has offered a general treatment of the stability of suspensions of particles in water.³² It was further stated that the present writers believe this theoretical treatment to be in error. Reiner's fundamental thought is that the conflicting forces in determining the stability or non-stability of a dispersion are cohesion, acting to cause aggregation of the particles, and adhesion, acting between the aqueous medium and the particles, tending to keep the particles dispersed.

He states that the work of separation incident upon increased dispersion of a microheterogeneous system is given by the expression

$$\frac{1}{2} ds(2\gamma A + 2\gamma B)^*$$

where ds is the increase of surface of the disperse phase, and γA and γB are respectively the "free surface energies" of the dispersed material and the dispersing medium. This formulation is incorrect. As has been pointed out the work of cohesion is given by the expression,

$$Wc = 2S\gamma_{AB}$$

where γ_{AB} is the free surface energy at the particle-dispersion medium interface. γA and γB do not enter the expression, because the only free surface energy changes in the aggregation and dispersion of particles immersed in an aqueous medium are respectively decreases and increases in the extent of the

* Reiner's actual expression is translated into the notation of this paper.

particle-dispersion medium interface. Therefore the only free surface energy involved is the corresponding free surface energy, namely γ_{AB} .

Reiner goes on to define the affinity of the particles for water as measured by the work of adhesion in terms of the Dupré equation,

$$W_A = \gamma_A + \gamma_B - \gamma_{AB},$$

where W_A is the work of adhesion and the other symbols have the same significance as in the previous equations. Without going into detail it may be said that the Dupré equation is not applicable to the aggregation and dispersion of particles of a single substance immersed in an aqueous medium. γ_A and γ_B do not enter the situation at all for the reasons indicated in the discussion of Reiner's equation for cohesion.

Reiner then says that the tendency for the aggregation of particles, or "attraction," is measured by the difference between the cohesion and adhesion, and that this is equal for unit area to the particle-aqueous solution interfacial free energy. Since the premises are not correct it would seem that there were no need for considering the conclusion further. It may be pointed out, however, that Harkins, Clark and Roberts⁵⁸ have measured the works of cohesion and adhesion for a large number of substances and the differences between the two by no means equal the free interfacial energies of the interface between the adhering substances. Reiner's result happens to be equal to the work of cohesion tending to hold particles together after they have made contact. The relation of the work of cohesion to the stability of dispersions of colloidal particles has been discussed at length in the present paper.

The Stability of Suspensions of Unsensitized Bacteria

The work of Northrop and De Kruif^{24,25} has been of great importance in the development of the theory of bacterial agglutination. The stability of the suspensions of the two types of bacteria which they studied varied markedly and regularly with the salt content of the dispersion medium. With salt concentrations below 0.001 molal both types regularly agglutinated when their electrokinetic potential was reduced below ± 15 millivolts. Under these conditions ± 15 millivolts was the critical potential. When the total salt concentration was raised to 0.1 molal, the suspensions were stable when the electrokinetic potential was reduced to much smaller values than ± 15 millivolts, in some cases even when it was reduced to zero.

Shortly after the work of Northrop and De Kruif, Loeb⁵⁹ showed that the stability of gelatin solutions is influenced by salts in an exactly similar way, and further that the stability of suspensions of collodion particles coated with surface films of gelatin also showed the same type of behaviour. Loeb⁶⁰ pointed out the similarity of his results of this type to those obtained by Northrop and De Kruif with bacteria. He concluded from his experimental work that the increased stability of the suspensions of protein-coated collodion particles in the higher salt concentrations was most probably due to increased affinity of their surfaces for water under these conditions. Later Oliver and Barnard⁶¹ and Netter⁶² also attributed the decrease in "cohesive force" of the surface

of cells by salts to increased affinity of the surfaces for water. It would seem highly probable on this basis that the increased stability of the bacterial suspensions of Northrop and De Kruif may also have been due to an increase in the hydrous condition of the surfaces of the bacteria in the higher salt concentrations. This probability also follows from the theoretical considerations which have been presented in this paper.

Since the suspensions were stable in some cases even when the electrokinetic potential was reduced to zero, it follows that the work of cohesion of the bacteria must have been reduced below its critical value. It was shown that the most probable cause for the reduction of the work of cohesion between particles dispersed in aqueous media, is an increase in the hydrous condition of the particle surfaces. Changes in salt concentration are well known to affect the state of hydration of hydrophilic colloidal particles, and hence very probably to alter their state of surface hydration.

Northrop and De Kruif clearly recognized that a decrease in "cohesive force" must have taken place as between their bacteria in 0.001 and 0.1 salt solution. As has been described in the first part of this paper, they devised and used an ingenious method for following changes in this value. They defined "cohesive force" by the values obtained by this method. There is some question as to whether the values obtained by them accurately expressed the value of the cohesive force as defined in this paper, because, for example, their method may well have involved work against viscosity in the separation of partially coalescent particles. Nevertheless, in any case, they definitely showed that a reduction in "cohesive force" as measured by their method always accompanied the phenomenon of suspension stability with electrokinetic potentials below ± 15 millivolts. This parallelism is convincing evidence that they were able to measure true cohesive force with sufficient accuracy to arrange their suspensions in the proper order with regard to their value, and that was the most important object of their "cohesive force" measurements.

It is apparent that the foregoing discussion does not add greatly to the conceptions of Northrop and De Kruif. Their work preceded the work of Kruyt's laboratory on the stabilizing action of hydration. Because of the more recent emphasis placed upon the hydration factors, it seems of value to indicate the relation of this factor to the results of Northrop and De Kruif. Briefly, we believe the hydration to be one of the factors which determines the "cohesive force" of Northrop and De Kruif and the critical value of the cohesive force.

Northrop and De Kruif further showed that if the salt concentration is increased well beyond 0.1 molal their bacterial suspensions again became unstable. There seems to be no question but that they were correct in attributing this to a "salting out" mechanism, that is to a dehydration and precipitation in the presence of a high salt concentration. The general conclusion from their results is that both electrokinetic potential difference and hydration are important factors in determining the stability of suspensions of the two types of bacteria studied by them, both being markedly affected by variations in the total salt content of the dispersing medium.

According to Northrop and De Kruif, the lowest concentrations of salts acted to affect the electrokinetic potential difference, medium concentrations to affect the "cohesive force" and still higher concentrations to affect the state of surface hydration. In the opinion of the present writers, it appears that the effect of the intermediate concentrations on "cohesive force" may also be interpreted as resulting from an effect on the hydration affinity of the bacterial surfaces.

The general indicated dependence of the stability of Northrop and De Kruif's bacterial suspensions upon both electrokinetic potential difference and hydration, clearly indicates that the surfaces of their bacteria resemble hydrophilic colloids. The similarity of behaviour shown by Loeb's collodion particles with gelatin surfaces is confirmatory evidence for this view.

Bacteria do not all have surfaces of this type, however. The interfacial technique of Mudd and Mudd^{9,10} has enabled them to study the relative ease of wetting by oil and water of the surfaces of a large number of different types of bacteria. They have found in this way that acid-fast bacteria are in general more readily wet by oil than by water, whereas non-acid-fast bacteria in general are much more readily wet by water. It would be expected on this basis that stability relations of suspensions of acid-fast bacteria would resemble those for hydrophobic colloidal particles rather than those for hydrophilic particles as found in the case of Northrop and De Kruif's bacteria.

Further we have cultures of three types of bacteria which have the exceptional property of forming stable dispersions in distilled water or acid less than 0.001 molal with electrokinetic potentials of the order of 0 to a few millivolts, i.e. well below the critical value of ± 15 millivolts operative in the case of the two types studied by Northrop and De Kruif, when the electrolyte content of the dispersion medium was less than 0.001 molal.

The general contention of Northrop and De Kruif was that the stability of their bacteria with very low electrokinetic potentials when the salt content was increased to above 0.1 molal, was that the increased concentration depressed the cohesive force of the bacteria. Since the above-mentioned three types of bacteria form suspensions which are stable in distilled water or very dilute acid, with electrokinetic potentials of 0 to a few millivolts, it is apparent that an extension of the views of Northrop and De Kruif is necessary to account for the stability of these suspensions.

According to the theoretical conclusions of the present paper it appears necessary that this type of stability is due to an extremely low work of cohesion due to surface hydration which is probably in excess of that obtaining in the case of the bacteria of Northrop and De Kruif. It is at least quite definite that the primary stabilizing action of hydration is operative over a wider range of conditions in the case of the three varieties described here. Acid-fast bacteria and the type just described represent extreme types selected from a large number of bacteria studied over a period of years on the basis of their wetting properties and cataphoretic behaviour. The two types seemed to offer splendid material for the extension of the general theory of the stability of bacterial suspensions.

Accordingly experiments have been performed to test the hypothesis that the stability of suspensions of acid-fast bacteria depend upon conditions more closely resembling those for the stability of dispersions of hydrophobic colloidal particles; and that the varieties with apparently markedly hydrous surfaces form suspensions whose stability depends more definitely upon the hydration factor, than do those of the suspensions of the two types of bacteria studied by Northrop and De Kruif.

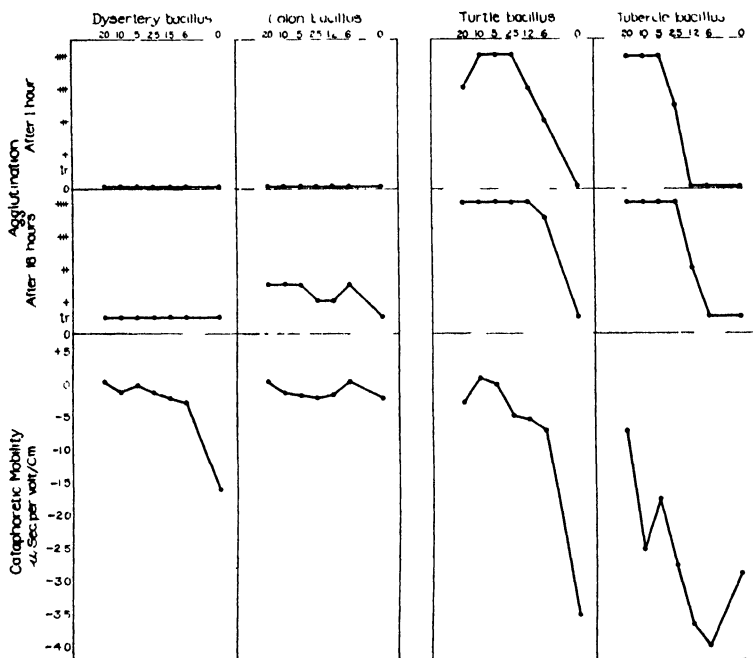


FIG. 2

Stability of hydrophilic bacilli and precipitation of hydrophobic bacilli in presence of acid. Washed bacteria suspended in solutions of HCl in distilled water. Abscissae, concentrations of HCl in millimols per liter. The hydrophilic dysentery and colon bacilli show little aggregation at any acidity. The hydrophobic turtle and avian tubercle bacilli show complete aggregation in acid concentrations which sufficiently reduce the electrokinetic p.d. To obtain electrokinetic p.d. in millivolts in this and subsequent figures multiply μ /sec. per volt/cm. by 12.6. (In this connection see Northrop and Cullen: *J. Gen. Physiol.*, 4, 638 (1921-22).

Fig. 2 records such an experiment. Washed suspensions in distilled water of two of the hydrophilic bacteria, the dysentery and the colon bacillus, and two acid-fast hydrophobic bacteria, the turtle bacillus and the Arloing strain of avian tubercle bacillus, were mixed with water and with dilute HCl solutions. The concentrations of HCl after mixing, in millimols per liter, are given as abscissae. Agglutination was read after one hour and after 18 hours in the ice box. The cathaphoretic mobilities were determined in a microcathaphoresis cell¹² following the 18 hours reading. Each suspension was examined in the cathaphoresis cell in HCl of the same concentration as that in which the agglutination readings had been made.

It is apparent that only a trace of agglutination of the dysentery bacillus occurred in any acid concentration, although the electrokinetic p.d. was extremely low; in 0.6 millimolar HCl the p.d. for the dysentery bacillus was about 3 millivolts. The colon bacillus showed very little agglutination although the p.d. was minimal; the colon bacillus was stable in distilled water with a p.d. of only about 3 millivolts. It is obvious that the stability of the colon bacillus in distilled water is neither attributable to reduction of the cohesive force by electrolytes nor to a high surface charge.

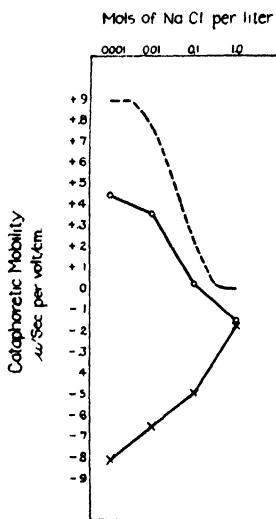


FIG. 3

Effect of salts on p.d. and lack of effect on agglutination of turtle bacillus and avian tubercle bacillus. Bacteria were suspended in 0.01 N HCl to which were added the amounts of NaCl indicated on the axis of abscissae. Circles, turtle bacillus. Crosses, avian tubercle bacillus. The upper broken line is the curve for typhoid bacillus in HCl and NaCl, redrawn from Northrop and De Kruif's²⁴ Fig. 4, p. 647. Unbroken line, complete agglutination. Broken line, no agglutination. The high electrolyte content inhibits agglutination of the typhoid bacillus, but not that of the hydrophobic acid-fast bacteria.

The turtle and avian tubercle bacillus, on the other hand, which in an oil-water interface show marked preferential wetting by the oil,⁹ are rapidly aggregated in concentrations of acid sufficient to reduce the p.d. below its critical value. In the case of the avian tubercle bacillus the value of this critical potential seems to be high, nearer that found by Powis⁶³ for oil drops than that found by Northrop and De Kruif for non-acid fast bacteria.

In Fig. 3 the same two acid-fast bacteria are set up in strongly acid solutions containing graduated concentrations of NaCl. The concentration of HCl after mixing was N/100 in each tube. The NaCl contents in the several tubes were 0.001, 0.01, 0.10 and 1.0 molar, respectively. These experimental conditions were chosen to duplicate as nearly as possible those of Fig. 4 in the paper of Northrop and De Kruif.²⁴ In our experiment the acid reduced the potential below the critical value for the hydrophobic bacteria and agglutination occurred in all tubes in spite of the very high electrolyte concentration. The corresponding curve in Northrop and De Kruif's Fig. 4 is replotted for contrast. With these bacteria no agglutination occurred until the very high "salting out" concentration was reached.

The general conclusions of this section of the paper may now be stated. Bacteria exist which display a wide range of surface types, from those which are markedly hydrophobic to those which are markedly hydrophilic. The factors governing the stability of dispersions of the various types in aqueous media are the same as apply to the stability of colloidal particles with similar types of surfaces. The theoretical considerations are those which have been described in a previous section.

Bacteria with strongly hydrophobic surfaces are stabilized in aqueous media chiefly by electrokinetic potential difference. They agglutinate when their electrokinetic potential is reduced below a definite relatively high critical value. Others, of the type studied by Northrop and De Kruif, form suspensions in which both electrokinetic potential difference and hydration are stabilizing factors of primary importance. This is not difficult to understand since evidence has recently been brought forward to indicate that the surfaces of many or most bacteria contain both hydrophobic and hydrophilic components.⁶⁴ Finally bacteria exist whose state of surface hydration is the primary stabilizing factor in their dispersions in aqueous media over a wide range of conditions.

The Stability of Suspensions of Sensitized Bacteria

"Sensitized" bacteria are bacteria which have combined with their corresponding antibodies. Sensitization results in marked changes in the physical properties of the bacterial surfaces, i.e. the sensitized bacteria are more cohesive, their wetting properties are altered, their electrokinetic p.d. is, under the conditions of the usual serological experiment, reduced, and their isoelectric point is shifted to a value near (but often not identical with) that of serum globulin.^{3,16} As was pointed out earlier in this paper these changes are consequent upon the formation of a surface deposit of antibody-globulin on the antigen. Since electrokinetic p.d., cohesion (and hydration), are the fundamental factors determining stability, the stability of sensitized would be expected to differ from that of unsensitized bacteria. As a matter of fact agglutination is the most familiar consequence of combination with antibody.

The remarkable specific chemical affinity between antigen and antibody enables the antibody in exceedingly high dilution to form an effective surface deposit on the antigen. Thus antisera may be prepared which agglutinate typhoid bacilli in a dilution of one volume of serum in a hundred thousand volumes of diluent. The surface deposit once formed, however, has many points of resemblance to deposits of serum proteins, egg albumin or other proteins formed by non-specific adsorption on bacteria or other particles. The non-specific deposit of serum proteins, in addition to requiring much higher concentration of protein to form an equivalent deposit, is in general less firmly held than the specific deposit.

In general with the progressive formation of a surface deposit the electrokinetic p.d. and isoelectric point of the particle approach those of the deposited substance. The stability conditions in the case of gelatin adsorbed on colloidal particles have been shown by Loeb closely to resemble these of gelatin solutions.⁶⁵ Loeb showed on the other hand that collodion particles coated with egg albumin showed the stability relations of denatured albumin rather than those of native albumin.⁶⁶ As was pointed out in the first section of this paper, students of specific bacterial agglutination from Bordet on have been impressed with the fact that sensitized bacteria were aggregated by traces of cations which were alike incapable of precipitating the unsensitized (non-acid fast) bacteria or the serum globulins with which the antibodies are associated.

The antibody-globulin combined with antigen has therefore been spoken of by Shibley¹⁸ and others as "denatured."

Northrop has discussed the general question of the change in the stability relations of suspensions of particles when their surfaces become coated with any variety of surface film. His discussion is on the basis of changes in electrokinetic potential difference and the changes in the "cohesive force."¹⁹ This same question may be discussed advantageously from the point of view of suspension stability presented in the present paper in a manner in which the general conceptions are very similar to those of Northrop.

A given dispersion of particles is stable either if the repelling force (electrokinetic potential difference) is greater than its critical value, or if the cohesive force of the particles ($2S\gamma AB$) is below its critical value. It follows from this that for aggregation and precipitation to occur both the electrokinetic potential difference must be below its critical value and the work of cohesion must be above its critical value.

A stabilizing or protective film forming substance is one that, under the conditions of test, results in a surface such that either the electrokinetic potential difference is above the critical value for that surface or that the work of cohesion of the surface is below its critical value, or both. A precipitating or sensitizing film forming substance is one such, that under the conditions of test, a surface results which is both below its critical potential and above its critical work of cohesion. It is apparent that one and the same substance may act either as a stabilizing or sensitizing film forming substance depending upon the conditions of test.

Since after combination with bacteria has occurred, the effect of antibody film on stability conditions is entirely analogous to that of other types of films, the above considerations apply to the agglutination of sensitized bacteria. It may immediately be stated that antibody films which cause agglutination of bacteria do so because they result in surfaces which are both below their critical potentials and above their critical works of cohesion under the conditions of test. It is misleading to state that the agglutination of bacteria by antibodies results "from a decrease in electrokinetic potential difference" or "from an increase in cohesive force."

Reiner's view that the sensitized surfaces are always less hydrous than the corresponding unsensitized bacteria, and that this difference in hydration is the fundamental change responsible for the agglutination of sensitized bacteria is obviously incomplete on the above basis. In this connection it will be remembered that it has been pointed out that dehydration of a given surface would be expected to lead to an increase in cohesive force. However, since in depositing an antibody film, we are forming an entirely new surface, this general relationship need not necessarily hold.

The conclusion of Tulloch¹⁹ and others that sensitized bacteria behave relatively more like denatured protein surfaces and that many unsensitized bacterial surfaces behave relatively more like normal protein surfaces, and Northrop and De Kruif's^{24,25} observations on the variation with the salt content of the medium of the cohesive force of these sensitized and unsensitized

suspensions both support the view that the sensitization of non-acid fast bacteria does give them surfaces which are less hydrated than in the unsensitized state.

However, several classes of carefully studied cells in the unsensitized condition show in an oil-water interface marked preferential wetting by the oil. After specific serum sensitization these same cells show marked preferential wetting by the aqueous phase of the interfacial preparations. Many acid-fast bacteria, notably the turtle and avian tubercle bacilli,¹⁴ red blood cells⁶⁷ and certain spirochetes⁶⁸ belong in this category. Sensitization in brief has been found by direct observation greatly to increase the difficulty with which water may be displaced by oil from the surfaces of cells of this class. It is reasonable to conclude that sensitization of these relatively hydrophobic cells has increased, rather than decreased, their surface hydration.

Yet sensitization of the hydrophobic cells of this class produces an increase in cohesiveness only slightly less striking than in the case of hydrophilic bacteria. This increase in cohesiveness may be directly observed in gross by the resuspension technique and microscopically by the interfacial technique. These instances afford clear examples of the fact that formation of a new surface as in sensitization, may result in increased cohesion, accompanied either by an increase or decrease in hydration, or at least in relative ease of wetting by water as compared to oil.

The interesting imitation by tannin of several manifestations of antibody action, first demonstrated by Reiner and his associates, makes it desirable to learn more about the effect of tannin on surfaces treated with it. We have studied the adsorption of tannin on a variety of substances. Mixtures of the test bacterial or other suspension were made with serial dilutions of tannin, the precipitates were washed and their cataphoretic mobility was determined in a microcataphoresis cell. It has thus been found that tannin forms an electronegative surface deposit, whose cohesion and precipitability by salts is somewhat greater than those of the untreated bacteria.

The detailed procedure was usually as follows:

A 10% solution of tannic acid (Merck's "Reagent") in distilled water was made. To one volume of this 10% solution an equal volume of 0.1 N NaOH was added. The pH of this mixture was usually from 6.0 to 6.7. Serial dilutions of this stock 5% neutralized tannin solution were made in 0.85% NaCl; dilution was usually in powers of 4, 1:4, 1:16, 1:64, etc. Equal volumes of the test suspension in 0.85% NaCl solution were added to the serial tannin dilutions. The series were kept overnight in the icebox and *agglutination* was read in the morning. All tubes were centrifuged and the supernatant fluids were decanted. A few drops of 0.85% NaCl solution were added to the sediment in each tube and these were shaken uniformly in a rack until the sediment in the control tubes was evenly suspended. The degree of aggregation of the treated sediments was recorded. These results are plotted as *resuspension*. Excess 0.85% NaCl was added to each tube and these were again centrifuged and the supernatant fluids decanted. The washed sediments were shaken up in 0.85% NaCl and studied in appropriate buffers in the microcataphoresis cell. In series in which serial dilutions of serum were used instead of tannin the subsequent procedure was the same.

The result of such an experiment is shown in Fig. 4. The test suspension in this case was a "rough" pneumococcus which was isoelectric at about pH 4.3. The bacteria were agglutinated and made more cohesive both by horse

immune serum and by tannin. The isoelectric point of the treated and washed bacteria was, however, shifted by the immune serum to a value of about pH 5.55, and by tannin the isoelectric point was shifted progressively toward the acid side to a pH below 1.8.

Another experiment is shown in Fig. 5. In this case a dysentery bacillus (Flexner type) was agglutinated by the serum of the patient from whom it was isolated and by tannin. This micro-organism had no measurable p.d. in M/50 acetate buffer of pH 4.4. Sensitization with the patient's serum gave

these bacteria an electrokinetic p.d. with isoelectric point at pH 5.4. Treatment with progressive concentrations of tannin gave the bacteria suspended in acetate of pH 4.4 an increasing negative p.d.

"Smooth" pneumococci, with their carbohydrate-rich capsules, were also

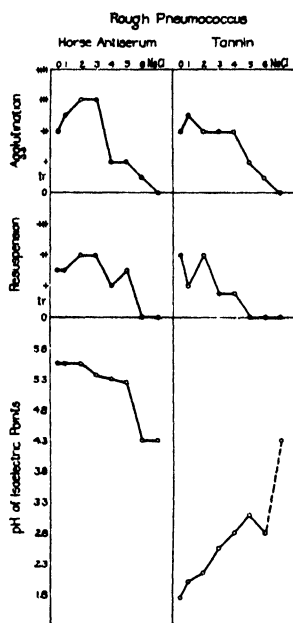


FIG. 4

The effects of specific immune serum and of tannin on the agglutination, resuspension (cohesiveness) and isoelectric point of a rough pneumococcus, isoelectric at pH 4.3. Bacilli treated with serum or tannin, washed, and studied in microcataphoresis cell. Abscissae dilutions, in powers of 4, of serum or of stock 5% neutralized tannin solution. i.e. 1 indicates four times diluted; 2 indicates 16 times diluted etc. In these experiments and in those illustrated in subsequent figures, all mixtures of bacterial suspensions with serum or tannin solutions contained equal volumes of the suspension and of the solution.

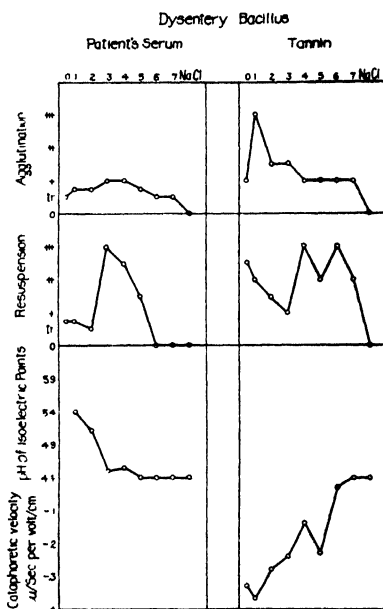


FIG. 5

The effects of specific immune serum and of tannin on the agglutination, resuspension (cohesiveness) and isoelectric point of a hydrophilic bacillus. Abscissae as in Fig. 4. The unsensitized bacillus was practically uncharged in buffer of any pH used. After sensitization with serum charge and isoelectric point near pH 5.4 were acquired. After treatment with tannin a negative charge was acquired.

agglutinated by tannin. Since these bacteria were electronegative even in acid solutions, however, little change in the electrokinetic p.d. as a result of adsorption of tannin was demonstrable.

The turtle bacillus proved very sensitive to agglutination by tannin. Suspensions of these bacilli were agglutinated by as little as 1 part in 100,000 of tannic acid or neutralized tannin. It would be difficult to suppose that agglutination of this hydrophobic bacillus was due to "dehydration."

With one exception all types of bacteria and other particles aggregated by strong tannin solutions were electronegative even in acid solutions. The exception was red blood cells which after treatment with strong tannin solutions developed isoelectric points in the neighborhood of pH 6.0. This result was very puzzling until it was observed that collodion particles added to these tannin-treated red blood cells promptly became isoelectric at about the same pH as the treated red blood cells. Collodion particles treated with tannin alone are strongly electronegative. It was thus evident that treatment with strong tannin solutions injured the red blood cells with liberation of some amphoteric substance which was readily adsorbed on the surfaces of the red cells themselves and of collodion particles. A similar source of error has been described by Abramson⁶⁹ for red blood cells in acid buffers and by Mudd and Mudd for white blood cells in acid buffers.⁷⁰

The resuspension observations plotted in Figs. 4 and 5 and also those plotted later in Fig. 6 and Fig. 7 refer to the relative ease with which the bacteria may be resuspended by mechanical agitation, after agglutination has proceeded for 18 hours and all suspensions have been centrifugalized. The difficulty of resuspension varies over wide limits, and while it frequently parallels the degree of agglutination, it may be partially independent of this value. Thus turtle bacilli are completely agglutinated both by their specific antiserum and by appropriate concentrations of acid. In the former case, they may be resuspended only with difficulty. In the latter resuspension is accomplished by very slight mechanical agitation.⁷¹ The fact that complete agglutination occurs in both cases, means that the work of cohesion exceeds the critical value in both cases. In the ideal case the resuspension test then furnishes a rough measure of the *extent to which the work of cohesion exceeds the critical value*. However in cases such as that of the agglutination of bacilli with specific antiserum it is probable that coalescence of surface antibody-films occurs on standing and centrifugalization after primary agglutination. The separation of coalesced particles is non-ideal and involves work against viscosity. The resuspension test thus measures composite differences in ideal work of cohesion combined with any differences which may exist due to different degrees of coalescence of different surfaces. In Figs. 4, 5, 6 and 7, the difficulty of redispersion parallels the degree of agglutination in all cases.

Since most bacteria are strongly electronegative at reactions approaching neutrality the usual result of serum sensitization is a reduction of electrokinetic p.d. The charge of the partially sensitized bacteria is a resultant of that of the bacteria themselves and that of the antibody-protein deposited

on the surface. The availability of three strains with negligibly small intrinsic surface charge offered the opportunity therefore to study the changes incident upon sensitization under somewhat simplified conditions.

In the experiments recorded in Figs. 6 and 7 the colon bacillus, dysentery bacillus and *S. pullorum* are sensitized each with a corresponding rabbit immune serum. In the experiment shown in the first column of each figure the bacteria were washed and suspended in M/14 acetate buffer of pH 4.4. The second columns show data with the bacteria washed and suspended in M/15 phosphate buffer of pH 7.3. In each case 1 cc. of bacterial suspension was mixed with an equal volume of each serial dilution of immune serum. The mixtures were incubated 2 hours at 37° and left overnight in the ice box. The agglutination was read in the morning; all tubes were centrifugalized, the supernatant fluids were decanted and a few drops of buffer were added to each tube. The tubes were shaken together in a rack and the results recorded as "resuspension." More buffer was then added, all

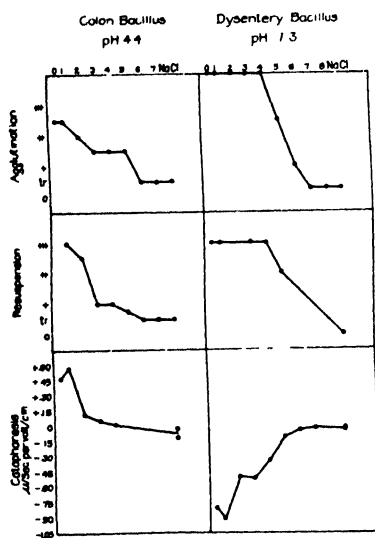


FIG. 6

The effects on agglutination, resuspension (cohesiveness) and electrokinetic p.d. of specific serum sensitization of hydrophilic bacteria. In the first column a strain of colon bacillus, suspended in M/14 acetate buffer of pH 4.4, is sensitized with rabbit immune serum. In the second column a dysentery bacillus (Flexner strain) suspended in M/15 phosphate buffer of pH 7.3, is sensitized with rabbit immune serum. Bacilli washed after sensitization and suspended in the corresponding buffers. Ordinates, intensities of reaction. Combination of antibody with antigen occurs on both sides of isoelectric point of antibody. Agglutination occurs parallel to increasing cohesiveness in spite of increasing charge, which may be either positive or negative.

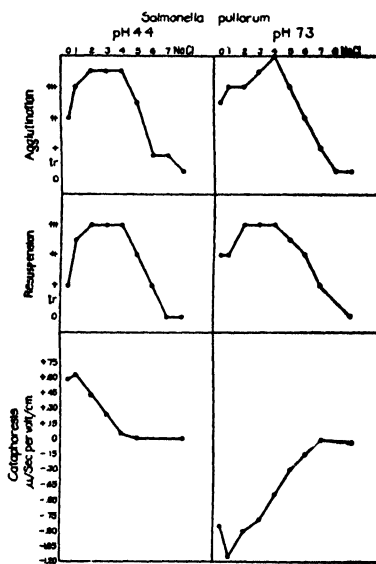


FIG. 7

The effects on agglutination, resuspension (cohesiveness) and electrokinetic p.d. of specific serum sensitization of hydrophilic bacteria. In the first column a strain of *Salmonella pullorum*, suspended in M/14 acetate buffer of pH 4.4, is sensitized with rabbit immune serum. In the second column the same bacillus, suspended in M/15 phosphate buffer of pH 7.3, is sensitized with rabbit immune serum. Experimental procedure and coordinates as in Fig. 6. Combination of antibody with antigen occurs on both sides of isoelectric point of antibody. Agglutination runs parallel to cohesiveness in spite of increasing charge. Note "agglutination prezone."

tubes were again centrifugalized, the supernatant fluids were decanted and the washed sediments were again shaken up in buffer. Microcataphoresis determinations were finally performed. Similar buffer mixtures to those used in making the original suspensions were of course used throughout the washing and cataphoresis determinations.

Figs. 6 and 7 show that antibody combines on both sides of its isoelectric point with antigen; this fact was already well known.^{72,73,74} They show, that in these cases agglutination was correlated with actual increases of electrokinetic p.d., the sign of charge being positive in the acid and negative in the acid buffer. Since the electrokinetic p.d.s throughout the whole experiment were within the critical potential zone for sensitized cells, this unusual correlation of decreased stability with increased charge is readily understandable. The decreased stability in these cases is evidently due to the substitution of cohesive, little hydrated antibody-protein surfaces for the strongly hydrated bacterial surfaces.

Attention is finally directed to the submaximal agglutination which occurred with the highest concentration of sensitizing serum in Fig. 7. This is the so-called "agglutination prezone" which is familiar to immunologists. A detailed discussion of this phenomenon would be outside the scope of this paper. It is worth pointing out, however, that this is not, as is sometimes supposed in uncritical treatments of the subject, due to the same mechanism as the zone phenomena observed in the mutual precipitation of positively and negatively charged colloids. For a critical study of the agglutination prezone the reader is referred to Shibley.⁷⁵

Summary

The first portion of the paper is devoted to an historical summary of the development of the present state of knowledge of bacterial agglutination.

A theoretical formulation of the factors affecting the stability of dispersions of colloidal particles in water and aqueous media is next presented. The treatment is not exhaustive, but rather aims to present a general statement of the modes of action and the interrelationships of the major physical factors governing stability, i.e. charge, cohesiveness (which can be expressed in terms of surface energy), and hydration.

A suspension of colloidal particles or of bacteria is considered to be stable, when either the repelling force, as measured by the electrokinetic potential difference, is sufficient to prevent actual contact of the particles when opportunity for their collision is presented in virtue of their Brownian motion, or the work of cohesion ($2S\gamma_{AB}$) tending to hold these together after contact is less than sufficient to overcome the tendency for their redispersion.

The undoubted importance of the state of hydration of colloidal particles is attributed primarily to the effect of the hydrous condition of the particle surface on the particle-dispersion-medium free interfacial energy (γ_{AB}), which in turn determines the work of cohesion between the particles, ($2S\gamma_{AB}$). The greater the state of hydration of a given surface, the more the surface resembles the aqueous dispersion medium and presumably the less the free inter-

facial energy. Therefore the greater the state of surface hydration, the less would be the cohesive force between the particles and the greater their tendency to form a stable dispersion. When a surface is altered by the deposit of another material upon it, however, no simple relation between the changes in hydration and cohesion so induced can be predicted. Certain errors are pointed out in the theory of suspension stability that has been formulated by Reiner.

In the next section, dealing with the stability of suspensions of unsensitized bacteria, it is shown experimentally that bacteria exist of a wide variety of surface types, from those which are strongly hydrophilic to those which are strongly hydrophobic. The factors determining the stability of a suspension of a given type of bacteria are shown to be those determining the stability of a dispersion of colloidal particles of the same surface type. The probable relation of surface hydration to the cohesive force of Northrop and De Kruif is pointed out.

In the final section, dealing with the stability of suspensions of sensitized bacteria, it is pointed out, on the basis of the aforementioned theoretical formulation, that the agglutination of bacterial suspensions by antibodies must be due to the fact that the antibody-globulin deposited on the bacteria by virtue of the specific combining affinity of antigen and antibody gives them surfaces which have both an electrokinetic potential difference below that of the critical value and a work of cohesion above the critical value for the antibody surface under the conditions of test. Formulations purely on the basis of "lowering of electrokinetic potential," "lowering of state of surface hydration," or increase of "cohesive force" are incomplete and misleading.

The last section gives the first experimental demonstration that adsorption of tannin on bacteria results in a surface of increased cohesiveness which is electronegative even in acid solutions. Tannin thus resembles antibodies in forming a cohesive deposit on the particles it precipitates; the tannin-treated surface, however, differs from the specifically sensitized surface in its electronegative character in acid solutions.

We are indebted to Dr. J. H. Northrop and to Dr. J. Freund for reading critically this manuscript.

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SOME APPLICATIONS OF COLLOID CHEMISTRY IN THE SERUM DIAGNOSIS OF SYPHILIS

BY HARRY EAGLE*

It is hardly necessary to point out the importance of the laboratory procedures used in the diagnosis of syphilis. The disease is characterized by long periods of latency, during which laboratory tests are the only indication of its presence; and its active manifestations may at times simulate those of other diseases so closely as to make a differential diagnosis impossible without the confirmatory evidence supplied by a serologic examination of the blood or spinal fluid.

Two types of test are generally used. Both involve the use of an alcoholic extract of normal tissue (e.g. beef heart) as "antigen." In the flocculation tests (Sachs-Georgi, Kahn, Müller, Meinicke, etc.), this extract is diluted with a small quantity of NaCl solution, forming a milky unstable suspension consisting of the lipoids of the antigen dispersed in the aqueous phase. If this suspension is added to normal human serum, the constituent particles remain (or become) dispersed, and there is no visible aggregation; but if it is added to the serum of a syphilitic patient, there is an agglutination of the lipid particles into visible aggregates, the formation of which constitutes a positive reaction. The second type of test is the well-known Wassermann reaction. As in the precipitation tests, the antigen is an alcoholic extract of animal tissue; but the diagnostic criterion is that a mixture of the antigen and syphilitic serum (or spinal fluid) develops the property of destroying a hemolytic component of fresh serum (complement).

The observations summarized in the following pages present no new facts or theories as regards the general field of colloidal chemistry; but they do constitute an interesting application of elementary properties of colloidal suspensions to a biological problem. Briefly, this problem is twofold: (1) What is the physical or chemical cause for the reactivity of syphilitic serum with normal tissue lipoids; and (2) how may the diagnostic tests based upon this reactivity be brought to maximum sensitivity and specificity?

I. The Cause for the Flocculation of Tissue Lipoids by Syphilitic Serum¹

If one drops the alcoholic extract of beef heart, hereafter designated simply as antigen, into an excess of water, one obtains a barely opalescent colloidal suspension consisting of finely divided lipid particles. The surface properties of these particles are summarized in Figs. 1 and 2. As determined both by cataphoresis and by the zone of least stability, their isoelectric point

* Aided by a grant from the Committee on Research in Syphilis, Inc.

¹ Figs. 1, 2, 3 and 4 are reproduced from J. Exp. Med., 52, 717 (1930) to which reference may be made for detailed protocols.

is around $\text{pH} = 2$, at which reaction there is a very slow agglutination into visible aggregates (solid portion of the curves). On the alkaline side of this isoelectric point, the particles are quite stable (broken portion of the curves). Despite the fact that $\text{N}/15$ NaCl suffices to reduce their cataphoretic potential to a small fraction of its original value, more than 20 times as much electrolyte is required in order to bring about their aggregation.¹ The stability of the particles is therefore not due solely to their negative electrical charge against water, but to an intrinsic hydrophilic property of the antigen lipid.²

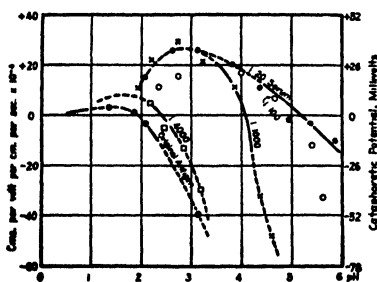


FIG. 1

The effect of hydrogen ion concentration and of normal serum upon the cataphoretic and flocculating properties of beef heart "antigen."

----- stable.
 ——— optically visible aggregation.

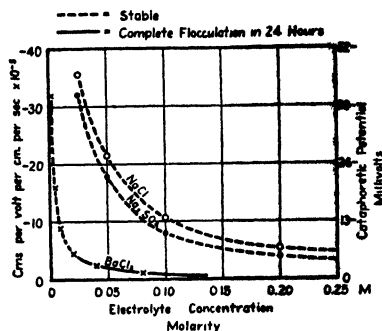


FIG. 2

Effect of electrolytes upon the flocculating and cataphoretic properties of "antigen" at $\text{pH} 6.0$.

In the presence of normal human serum these antigen particles adsorb serum protein, which forms an incomplete film around the lipid particles. As more and more protein is adsorbed, the zone of optimal precipitation, coinciding with the cataphoretic isoelectric point, gradually shifts to a more alkaline reaction, approaching as its maximum value $\text{pH} = 5$, the isoelectric range of serum protein, at which point the particle is presumably completely covered by the adsorbed film (Fig. 1).

The significant feature of this adsorption is that on either side of this isoelectric point, the particles remain stable; the electrolyte concentration required to produce flocculation of the sol is not affected. Clearly, the adsorbed protein retains its water-soluble, hydrophilic properties, acting as a protective rather than a sensitizing film, and preventing even the normal flocculation of the lipid particles at their original isoelectric point.

Quite different relationships obtain if syphilitic serum is added to the suspension. The constituent particles aggregate and sediment, leaving a

¹ In a sol containing 0.08% lipid. The exact coagulation value depends upon the method of preparation of the antigen, the method used in preparing the sol, its concentration, the hydrogen ion concentration, the quantity of sensitizing material (see page 700), etc.

² The active lipoids in a tissue extract are insoluble in acetone. We have found that hydrolysis of the extract by HCl yields fatty acids, and water-soluble substances containing P and N. Presumably, therefore, lecithin constitutes the bulk of the lipoids; its numerous water-soluble groups would explain its hydrophilic properties. Since the activity of the lipid in the serological tests is destroyed by this acid hydrolysis, as well as by prolonged saponification, it would seem that the whole molecule is necessary for serological activity.

clear supernatant fluid. If this precipitate is washed free of serum, resuspended in water, and the surface properties of its constituent particles examined, one finds that, as in normal serum, their isoelectric point is intermediate between that of serum protein and the original value of $\text{pH} = 2$; at this isoelectric point there is a rapid clumping (Fig. 3). The distinguishing property of these sensitized particles, however, is that away from this isoelectric point, the stability of the particles is determined solely by the mutually repellent surface charge. As soon as this is repressed below a certain critical

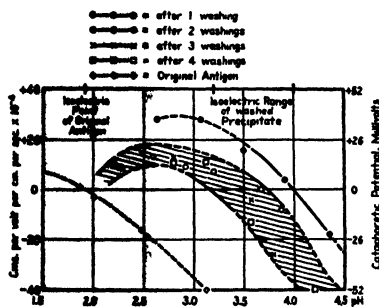


FIG. 3

Effect of hydrogen ion concentration upon the cataphoretic and flocculating properties of antigen after contact with syphilitic serum.

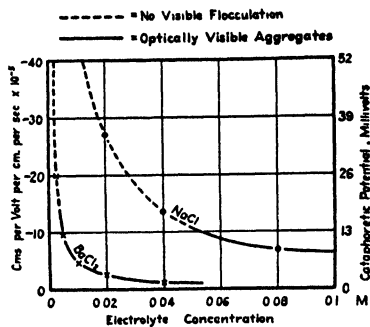


FIG. 4

Effect of electrolytes upon the flocculating and cataphoretic properties of the lipid particles after contact with syphilitic serum.

level (10-15 millivolts), as by $N/20$ NaCl, the particles cohere to form visible aggregates (Fig. 4).

"Syphilitic" protein can be demonstrated in the washed precipitate both chemically (Klostermann and Weisbach;¹ Scheer²) and immunologically (Otto and Winkler³); and the specific substance in syphilitic serum is constantly associated with the globulin fraction of the serum protein (Gloor and Klinger;⁴ Sahlman;⁵ Kapsenberg;⁶ Stern;⁷ Eagle⁸). Moreover, heating a suspension of these sensitized particles at 100°C coagulates and separates a protein film, so that one obtains particles of heat-coagulated specific protein side by side with unchanged, stable lipid particles which can combine afresh with syphilitic serum (Georgi;⁹ Eagle¹⁰).

¹ Deutsch. med. Wochenschr., 47, 1092 (1921).

² Münch. med. Wochenschr., 68, 43 (1921).

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⁴ Z. Immunitätsforsch., 29, 435 (1920).

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⁸ J. Exp. Med., 52, 717 (1930).

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¹⁰ J. Exp. Med., 52, (1930).

It would therefore appear that syphilitic serum contains a protein with a strong specific affinity for these lipid particles, quite different from the attraction determining the non-specific, loose adsorption of normal serum protein. The irreversible combination of this particular protein with the antigen particles in some way deprives it of its hydrophilic properties. Instead of forming a protective, water-soluble film, it forms a film of "denatured" water-insoluble protein which is as unstable as e.g. globulin coagulated by heat, and which sensitizes the underlying lipid particle to agglutination by electrolyte.

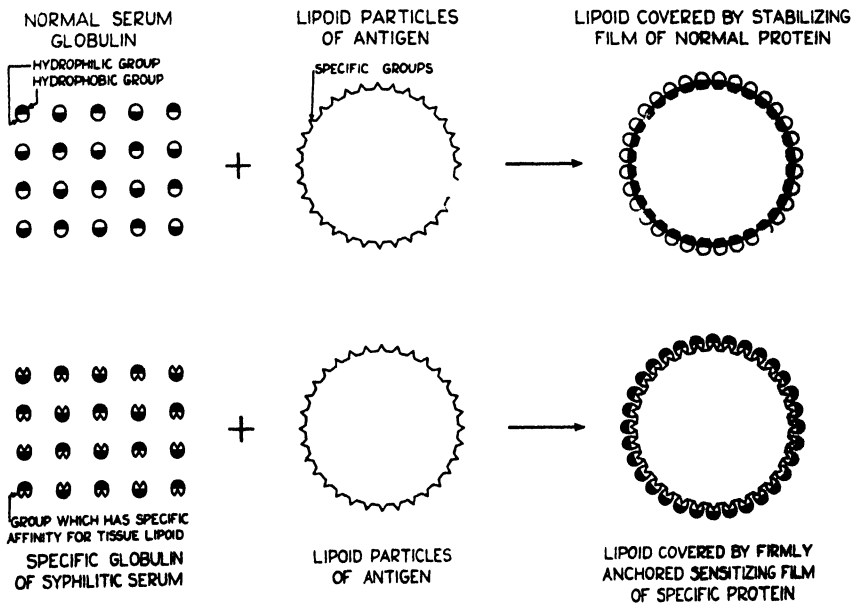


FIG. 5

Hypothetical explanation for the "denaturation" of the specific antigen-globulin at the surface of the lipid particles.

Without going into the confusing technical details of the Wassermann reaction, it may be stated that the same film which causes precipitation is also responsible for the phenomenon of complement fixation (Eagle¹).

I can only venture a guess as to the cause for this irreversible change in the specific globulin when it combines with the antigen lipid. Possibly the complex protein molecule is polar, containing both hydrophilic and hydrophobic groups, the former determining its solubility in water. When normal protein is adsorbed by the lipid particles the hydrophilic groups would naturally face the water phase, and the particle as a whole would have the stable surface properties of a dissolved protein. Perhaps in the active protein of syphilitic serum the hydrophilic groups have an even greater specific affinity for the lipid than they do for water. In such a case, following the combination of the

¹ J. Exp. Med., 52, 739 (1930).

lipoid with the protein, the residual hydrophobic groups of the protein molecule would necessarily face the water phase, endowing the complex with the surface properties of "denatured," water-insoluble protein, and precipitation would naturally follow.

This hypothesis, and I wish to emphasize the fact that it is only an hypothesis, as yet not amenable to experimental test, is diagrammatically illustrated in Fig. 5.

Altogether aside from the truth or falsity of this particular theory, it is highly significant that the aggregation of antigen lipoid by syphilitic serum is entirely analogous to the aggregation of bacteria, red cells, or dissolved protein by the homologous antiserum. It suggests the possibility that the active protein in syphilitic serum represents an antibody to products of syphilitic infection, presumably the *Treponema pallidum*.

II. The Explanation of the Sensitizing Action of Cholesterol¹

It has been known for many years that the addition of cholesterol to an alcoholic extract of normal tissue causes a very great increase in its sensitivity in the diagnostic tests for syphilis, despite the fact that cholesterol as such is quite inactive. We believe the following to be an adequate explanation of this phenomenon.

When the cholesterolized antigen is dropped into an excess of NaCl N/7, it forms a stable colloidal suspension similar to that formed by antigen alone, but somewhat more opalescent. Many more particles are visible by dark field examination, but there are no coarse sedimenting aggregates such as appear if an alcoholic solution of cholesterol is dropped into salt solution.

This effect of the tissue lipoids in causing a stable colloidal dispersion of hydrophobic cholesterol is considered analogous to the detergent action of soap upon particles of dirt. When the alcoholic solution of tissue lipoids and cholesterol is diluted with water, the immediate tendency of the cholesterol is to cohere into coarse crystalline sedimenting aggregates, exactly as it does when its alcoholic solution is dropped into water. While the cholesterol particles are still of colloidal dimensions, however, it is believed that they adsorb the surface-active lipoids of the antigen, which have a lower interfacial tension against water and which act as a protective colloid, preventing any further cohesion. The suspension remains of colloidal dimensions and therefore stable. The antigen-covered cholesterol particles are now to all intents and purposes particles of pure antigen (Fig. 6).¹

There is ample confirmatory evidence that this is the cause of the stable suspension formed by a cholesterolized antigen. The isoelectric point of the particles it forms is around pH2, the same as that of antigen particles, and approximately M/1 NaCl is necessary to bring about their flocculation in a dilute suspension. In marked contrast, cholesterol particles have no definite isoelectric point, and are so hydrophobic that they precipitate even in water, despite a very high surface charge (> 100 millivolts). Many other hydrophobic

¹ Figs. 6 and 7 are reproduced from J. Exp. Med., 52, 747 (1930).

substances, which normally form coarse aggregates when their alcoholic solution is dropped into water, are brought into colloidal "solution" by the addition of tissue lipoids to the alcoholic solution. Conversely, many other surface-active substances, such as serum, Na-oleate, Na-taurocholate, etc., cause a colloidal dispersion of cholesterol if its alcoholic solution is dropped slowly with shaking into an aqueous solution of these surface-active substances.

The more cholesterol is added to the antigen before diluting with saline, the coarser are the individual particles of the sol obtained, the more are visible by microscopic examination, and the greater is the opacity of the sol. Presumably, this is due to the fact that the aggregation of the cholesterol is more rapid at higher concentrations, allowing the aggregates to become larger before they adsorb a completely protective film of antigen-lipoid (Fig. 7).

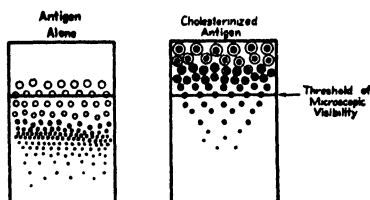


FIG. 6

Diagrammatic representation of the effect of cholesterol in causing a coarsened dispersion of the antigen sol.

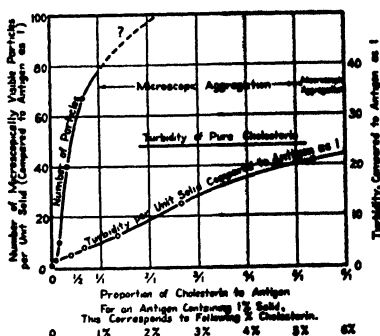


FIG. 7

Effect of cholesterol upon the turbidity and the number of microscopically visible particles in an antigen sol.

The effect of cholesterol is increasing the sensitivity of the antigen, illustrated in Fig. 7, is not due to the cholesterol as such, but to the coarsened dispersion of the antigen-lipoids which it causes. For obvious reasons the larger particles formed by a cholesterolized antigen are easier to agglutinate into visible aggregates. Moreover, although there is no satisfactory explanation for the observation, it can be shown that such large particles have a much greater affinity for the specific protein of syphilitic serum than similar particles of smaller size. Thus, if one drops antigen into an excess of saline, one obtains a finely dispersed, barely opalescent lipid sol: if, using the same quantities, one drops the saline to the antigen, a much coarser solution is obtained, similar to the solution produced by a cholesterolized antigen. Despite the fact that the particles of the former sol have a much greater total surface area, the latter is far more effective in the Wassermann reaction. The greater sensitivity of the cholesterolized antigen in both the precipitation and complement fixation tests is therefore believed to be due, in part at least, to the fact that the average particle in the aqueous dilution is larger than the particles formed by pure antigen.¹

¹ An additional factor may be an orientation of the adsorbed lipid molecule on the surface of the cholesterol, presenting specific reacting groups to the aqueous phase.

III

We now come to an important practical application of the foregoing discussion. If cholesterol owes its activity solely to the fact that it causes a coarser dispersion of the antigen lipid by acting as an inert core onto which the active lipid is adsorbed, then (1) it should be used up to the limit of its solubility in alcohol, and (2) any substance which because of a lower surface tension against antigen lipid than against water, would adsorb the antigen lipoids in an aqueous phase, should have a similar sensitizing action.

A large series of water-insoluble, alcohol-soluble substances were tested; and with only a few exceptions they all had qualitatively the same effect as cholesterol. Added to the alcoholic extract, they caused a coarsened dispersion of the antigen lipid when the extract was diluted with water; and when this dilution was tested for its sensitivity in the Wassermann reaction, it was found to be more sensitive than the original antigen. Some of the substances are grouped in Table I in the order of their sensitizing efficiency.¹

The use of the best of these sensitizers as adjuncts to cholesterol has enabled us to develop a Wassermann antigen which is considerably more sensitive than any hitherto available. The particular substances used are sitosterol and a sterol derived from wool.

TABLE I²

Sensitizing Efficiency of the Various Alcohol-Soluble, Water-Insoluble Substances tested

Slight	Fair	Very good
Gum guaiac	Gum balsam	Cholesterol
Gum sandarac	Gum copal	Sitosterol
Salol	Gum mastic	Wool Sterol
Gum shellac	Gum elemi	Corn Germ Sterol
Resin	Tolu balsam	Gum thus
Benzoin	Benzophenone	Safrole
Terpineol	Aurin	Diphenyl
Benzil		Dimethyl naphthylamine
<i>p</i> -iodobenzene		Methyl stearate
Ethyl- <i>m</i> -nitrobenzoate		Methyl palmitate
β -Chloronaphthalene		
Triphenyl phosphine		
<i>d</i> -Naphthonitrile		
<i>p</i> -nitrobromobenzene		
Ethyl palmitate		
<i>p</i> -Bromotoluene		

¹ Possibly, the sensitizing efficacy of these substances depends upon the magnitude of the expression

$$\frac{\text{interfacial tension}}{\text{substance} \times \text{water}} - \frac{\text{interfacial tension}}{\text{antigen} \times \text{water}} - \frac{\text{interfacial tension}}{\text{substance} \times \text{antigen}}$$

The greater this difference, the more avid would be the adsorption of the active lipid by the microscopic aggregates of the sensitizer. Unfortunately, the first term cannot as yet be determined experimentally.

² Reproduced from J. Exp. Med., 53, 605 (1931).

Flocculation tests, because of the simplicity of reagents and of technique, as contrasted with the laborious complexity of the Wassermann reaction, would greatly simplify the serological diagnosis of syphilis were it not for the too great personal factor involved in the interpretation of end results with minute degrees of aggregation. The addition of corn germ sterol in the proper proportions to a cholesterolized antigen to a great extent removes this objection. A new flocculation test based on its use has been devised and is now in routine use at the Johns Hopkins Hospital. The apparent superiority of the test is considered to be due to the fact that when this antigen is diluted with salt solution, it forms a colloidal suspension consisting of comparatively large needle crystals of sterol covered with a film of the active lipid, instead of the minute amorphous particles formed by a cholesterolized antigen. Despite the fact that these needles are sufficiently large to be seen as a cloud of refractile particles when the suspension is shaken, the mixture of normal serum and antigen is extremely stable, remaining translucent and showing no gross sedimentation or microscopic aggregation even after 24 hours. Upon addition to syphilitic serum, however, the crystals combine with the specific protein as already described, and subsequently clump to form granular aggregates of crystals readily visible to the naked eye and under the microscope; upon centrifugalization, these form large coherent clumps. Properly performed, the test is very sensitive, and allows for a sharp differentiation between syphilitic and normal serum.

Summary

1. Observations on the aggregation of an aqueous suspension of beef heart lipoids (antigen) by syphilitic serum indicate that the phenomenon is wholly analogous to the aggregation of bacteria, red cells or a dissolved antigen by the homologous antiserum. The mechanism of the reaction is conceived to be as follows. A specific component of syphilitic serum, associated with the globulin fraction of the serum protein, is firmly bound onto the surface of the individual particles of the lipid sol, forming a sensitizing film of denatured hydrophobic protein quite unlike the stabilizing film formed by adsorbed normal serum protein. The original particles, immersed in water or normal serum, are stable because of an inherent hydrophilic property of the surface; while the stability of the sensitized particles is determined by their charge against water. When this mutually repellent charge is depressed below the critical value (10-15 millivolts) by electrolytes, the particles cohere to form optically visible aggregates.

The same film which causes aggregation also adsorbs complement, giving the Wassermann reaction of complement fixation.

2. This complete analogy to the truly specific antigen-antibody reactions suggests that the reactivity of syphilitic serum with normal tissue lipoids may be due to the presence in such serum of antibodies to as yet uncharacterized products of infection.

3. The effect of cholesterol in increasing the diagnostic efficiency of the antigen is considered due to the fact that when the cholesterolized antigen is

dropped into water, microscopic aggregates of cholesterol in statu nascendi adsorb the serologically active antigen lipoid, forming particles which are many times larger than those formed by the antigen lipoid alone. This coarser dispersion is much easier to agglutinate; moreover, the larger particles have an unexplained greater affinity for the specific component of syphilitic serum. Both these factors would account for the greater sensitivity of the cholesterolized antigen in the diagnostic tests.

4. This explanation of the sensitizing action of cholesterol led to the search for substances which might have a similar affect. Of the numerous such substances found, sitosterol and sterols derived from corn germ and wool have proven of practical importance. Their use as adjuncts to cholesterol causes a significant increase in the sensitivity of the Wassermann antigen; while the physical properties of the corn sterol have led to the development of a new flocculation test for syphilis of great simplicity and sensitivity.

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THE COUPLED NATURE OF LACTIC ACID-GLYCOGEN SYNTHESIS IN MUSCLE

BY DEAN BURK*

The view developed so largely by Meyerhof and Hill that the synthesis of glycogen or carbohydrate from lactic acid in isolated muscle requires energy derived from the oxidation of such compounds has been questioned recently by Bancroft and Bancroft,¹ who have attempted to demonstrate that the formation of glycogen from lactic acid can be explained equally well upon other grounds. They suggest that a reversible equilibrium exists between the two compounds which may be disturbed by extraction of glycogen from solution by adsorption on muscle protein, thereby causing further formation of glycogen. This implies that the free energy of the synthesis is small, or at times zero.

It will be shown here that the Bancroft and Bancroft reversible equilibrium explanation is quantitatively inconsistent with the existing available thermodynamic free energy data.² Although aware that "the equilibrium point of this reaction is well over on the lactic acid side" it would appear that these writers failed to appreciate the quantitative completeness of the spontaneous breakdown, as will be evident immediately upon consideration of the free energy data.

From data given elsewhere,³ in a form somewhat different⁴ from that employed here, however, the free energy of the following synthesis, as it is normally considered to occur in muscle,

lactate ion (0.002 M = 0.018%) + H⁺ (2.5 × 10⁻⁸, or pH 7.6) = *n* glycogen (1%) (1 is 393 cal/gm., or 35370⁵ cal/mol of lactic acid (the heat of reaction is 268 cal/gm., or 24120 cal/mol). Correspondingly, at pH 3.28, where the free

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¹ J. Phys. Chem., 35, 194 (1931).

² Bancroft and Bancroft state: "The extent of this coupled reaction is remarkable, for Hill has shown that under suitable conditions the ratio of the amount oxidized to the amount synthesized is one to five or one to six." The ratio actually corresponds to an efficiency of 100 × (5/(325000/35370)), or 46%, where - 325000 cal. is the approximate free energy of combustion of lactic acid under physiological conditions. As a matter of fact, the writer has recently indicated (J. Phys. Chem., 35, 432-56 (1931)) that the coupled autotrophic reduction of carbon dioxide by hydrogen has an efficiency of substantially 100% when (as in the glycogen synthesis just considered) the extraneous maintenance energy of the biochemical machine accomplishing the reaction is neglected. Several other fairly highly efficient reactions were likewise discussed, and also a considerable number of reactions with efficiencies of 20-50%.

³ Burk: Proc. Roy. Soc., 104B, 153-170 (1929).

⁴ Entropy changes rather than free energy changes received chief expression.

⁵ In accordance with convention, the accuracy of free energy values is not indicated by the number of significant figures given.

energy of neutralization is zero at the concentration of lactic acid considered, the free energy of the synthesis

$$\text{lactic acid (0.002 M)} = n \text{ glycogen (1\%)} \quad (2)$$

is 336 cal/gm., or 30240 cal/mol of lactic acid (the heat of reaction is - 180 cal/gm., or 16200 cal/mol). n is the reciprocal of the ratio of the molecular weight of glycogen to that of lactic acid, and its significance will be considered later.

Bancroft and Bancroft's statement "From a purely chemical point of view this reaction (lactic acid-glycogen synthesis) should not require much energy" is obviously untrue; on account of the very large free energy requirement (a positive value of 35370 cal.) it is inconceivable that reaction (1) as written could occur spontaneously in muscle, at least in experimental, physiologically significant quantity. The question might remain, however, as to how far Bancroft and Bancroft would agree that Equation (1) represents approximately the essential conditions, particularly of concentration, of the reaction taking place in muscle.

They affirm (within ± 10 -fold) the adopted and generally accepted concentration of 0.002 M lactic acid dissolved in the plasma of fresh, isolated muscle.¹ It may be pointed out that for every 10-fold dilution or concentration of lactic acid the free energy of Equation (1) becomes changed respectively ± 1365 cal., i.e., changed to 36735 or to 34005 cal., respectively. Correspondingly, for every 10^{\pm} - fold dilution or concentration, the free energy becomes changed by approximately ± 1365 cal. Hence for equilibrium conditions to prevail (i.e., for ΔF to equal zero) the activity (or approximate concentration) of lactic acid would have to be maintained at the impossible figure of $0.002 \times 10^{(35370/1365)}$ M, or ca. 10^{23} M. Even neglecting neutralization, which normally takes place more or less completely under physiological conditions, and considering glycogen formation according to Equation (2), as might occur independently of muscle, the figure remains still as high as $0.002 \times 10^{(30240/1365)}$ M, or ca. 10^{20} M.

Likewise, it can easily be shown that adoption of physiological pH values other than 7.6 would influence the figure of 35370 cal. in Equation (1) but relatively little. At pH 6.9, for instance, it is reduced by only 990 cal. to 34380 cal.

With respect to the concentration of glycogen, Bancroft and Bancroft have introduced one new factor hitherto considered but little. They assert (as may very well be the case) that the 1% glucogen in muscle does not exist in solution but is almost completely adsorbed on muscle protein, the adsorption being reversible. In other words, the concentration of glycogen to be reckoned with in Equation (1) is not 1%, but something like 0.01%, or even less, i.e., there obtains an adsorption of 99% or more. They state qualitatively, "the reaction can be forced back from lactic acid to glycogen by the adsorption of glycogen out of solution on the protein, thereby reducing the amount of free glycogen in solution and causing the formation of more to re-

¹ This value would in their view represent the approximate normal equilibrium concentration of lactic acid in the muscle system at rest.

establish the equilibrium." However, as shown in the manner above with lactic acid, a physiologically inconceivable, great reduction in the concentration of free glycogen would be required, $10^{(35370/1365)}$ -fold, or 10^{26} -fold, even granting for the moment that the value of n in Equation (1) is unity. Assuming a value of $n = 1/1000$, the concentration reduction required would be $10^{(35370/1365)}$ -fold, or ca. 10^{26000} -fold, to $10^{-26000}\%$. As shown before (*loc. cit.*), owing to the very small value of n ($1/100$ to $1/1000$), the free energy of dilution of glycogen is substantially zero for all physiological concentrations, as may be seen from the formula:

$$\Delta F_{dil} = n RT \ln(x\%/y\%) = 1.365 \log(x\%/y\%). \quad (3)$$

where y is the physiological concentration to be considered, and n is taken to be $1/1000$. Thus, where $y\%$ is 0.001% (instead of 1%), ΔF_{dil} becomes only -4 cal. This 1000-fold dilution of glycogen decreases 35370 by only $100 \times (4/35370)$ or ca. $.01\%$, leaving reaction (1) substantially as spontaneously unversible as before. Therefore the reversible equilibrium explanation is in disagreement not only with existing free energy data, but also with *a priori* mass law considerations of the relative molecular weights of glycogen and lactic acid. This *a priori* argument is not necessarily so important, however, since the problem of sugar synthesis from lactic acid involves a large positive free energy in the same way that glycogen synthesis does, and yet in sugar synthesis n is $1/2$, i.e., not far from unity.

As a corollary to the above reasoning it follows that the free energy of glycogen adsorption on protein is totally insufficient to account for the synthesis, since the adsorption¹ is never complete enough, i.e., it is never so complete that about less than 10^{-25999} mg. glycogen per gram of muscle plasma is unadsorbed. Moreover, as just pointed out with respect to free energy relationships, sugar synthesis is similar to glycogen synthesis qualitatively and quantitatively, so that for sugar synthesis to take place (in the absence of simultaneous glycogen synthesis) postulation of adsorption on protein and removal from solution would likewise be required, as in the case of glycogen synthesis. Whether such adsorption takes place to a great extent in the ordinary sense is questionable; in the sense required by calculations similar to those given with respect to glycogen, it unquestionably does not take place.

It should be recalled that refutation of the reversible equilibrium explanation had been accomplished more or less successfully by Meyerhof and others on the basis of heat of reaction, rather than free energy of reaction, data. Obviously, however, a critical and ultimate decision must rest upon free

¹ The adsorption is of course itself a reversible process, according to Bancroft and Bancroft, so that the calculations of the previous paragraph with respect to glycogen concentration could be based upon either that amount in free solution or that amount adsorbed with exactly the same results or final conclusions. Since, however, in order to employ the latter method, the free energy of adsorption would have to be known (and added to that of Equation (1)) it is obviously much more convenient, and in the present state of knowledge essential, to proceed as has been done.

energy data.¹ The calculations required to produce the data given here were carried out with considerable precision; so far as the writer is aware, no assumptions were employed which if arbitrarily but judiciously changed would greatly decrease or enhance the large positive free energy value of lactic acid-glycogen synthesis in muscle given.

Bancroft and Bancroft concern themselves more with the details of glycogen-lactic acid breakdown than with lactic acid-glycogen synthesis. Owing to the long-established spontaneous nature of the breakdown, the really deciding test of the freely reversible equilibrium explanation must now concern itself with the synthesis; for this reason the present paper is considering chiefly the synthesis.² It may be pointed out with respect to the breakdown, however, that if lactic acid were in equilibrium with glycogen just before a muscle at rest were stimulated, the free energy of the breakdown subsequently called forth by stimulation would, per mol of lactic acid, be so close to zero as to be totally incapable of accounting for the work performed in any contraction of appreciable duration. So far as is known at present, the anaerobic performance of mechanical work derives its free energy *ultimately* from glycogen-lactic acid breakdown; this statement needs no qualification with respect to the finding within the last year or two that a certain amount of mechanical work may be obtained under conditions where lactic acid formation is prevented by iodo-acetic acid poisoning. Although other reactions may even under normal conditions be more immediately responsible for the performance of mechanical work than glycogen-lactic acid breakdown, presumably the latter is normally required later to reverse the other reactions so that they may then re-perform work.

Bancroft and Bancroft state: "If this is in reality a coupled reaction, one should be able to take lactic acid, oxidize it in the presence of protein, and form glucose." This is not necessarily true; one or more of the intermediate processes may require enzymatic catalysis. A coupled reaction need not, by

¹ It is interesting to note, also, that upon the basis of the free energy data given in this paper, the possibility suggested by Kluyver (Archiv. Mikrobiol., 1, 181 (1930)) is likewise precluded, that in glycogen synthesis from lactic acid the mechanism proceeds by way of pyruvic acid and acetaldehyde. In such an event, from stoichiometric considerations alone, no more than two molecules of lactic acid could disappear in synthesis, per one molecule burned, whereas (see p. 2) theoretically ten are possible, and experimentally five have been observed. The theory of the mechanism involving passage through a 2-carbon molecule stage (such as acetaldehyde) can not be true, therefore. Escape from this conclusion can involve only the hypothesis that the numerous, and variously performed experimental measurements of the ratio have been incorrect, or wrongly interpreted.

² It is beyond the scope of this paper to trouble to prove that not only does lactic acid-glycogen synthesis in muscle require a large amount of free energy but also that this free energy is supplied by oxygen consumption. It need scarcely be mentioned that no anaerobic reaction in muscle is known capable of providing such a large amount of free energy for the large amounts of synthesis which may on occasion take place. Although not inconceivable that very small, substantially unmeasurable amounts of synthesis might take place through energy provided by some anaerobic reaction, possibly incidental to the mechanism, this would be beside the point, since we are interested here in explaining the large synthesis transformations observed experimentally.

Bancroft and Bancroft state: "... the oxidation of lactic acid is not coupled with the synthesis of glycogen, but occurs simultaneously in the presence of oxygen," but offer no explanation for the fact that synthesis does not occur in the absence of oxygen. Likewise they do not explain, even on the basis of their theory, how stimulation causes elution of glycogen from protein.

its very nature, invariably proceed in the absence of an enzyme. Exception must be taken to Bancroft and Bancroft's conception of coupling as excluding enzymatic action, at least so far as the term coupling has been employed by physiologists with respect to the synthesis under discussion.

In view of Equations (1) and (2), it is not at all surprising that Bancroft and Bancroft failed in an experimental attempt to show that glycogen could be formed from lactic acid when "Using 15 cc. of a .2% solution of d-lactic acid, 10 cc. of M/2 KH_2PO_4 , and 10 cc. of enzyme solution; and approximately 30 grams of egg white. . . ." In any such future attempts, great caution will have to be exercised in evaluating positive results, i.e., in excluding all other possible reactions, particularly oxygen consumption, which might be responsible for providing the necessary free energy or equilibrium point shift. Indeed, on account of the great heterogeneity of the system in which the synthesis would have to be carried out, the formation only of considerably more than traces of glycogen could give rise to the suggestion that the thermodynamic data given here are inadequate or inapplicable.

Summary

1. The theory recently suggested by Bancroft and Bancroft that in muscle glycogen may be synthesized from lactic acid according to a freely reversible shift in the equilibrium point caused by adsorption of glycogen out of solution is shown to be quantitatively inconsistent with both (1) the existing thermodynamic free energy data and (2) *a priori* mass law considerations. The theory is also shown to be unable to account for the production of mechanical work in muscle upon the basis of free energy derived from glycogen-lactic acid breakdown.

2. The failure of Bancroft and Bancroft to accomplish the synthesis experimentally *in vitro* is in accord with the prediction of thermodynamic data given in this paper, according to which one milligram of glycogen would form spontaneously in not less than some billion trillion liters of a .2% solution of lactic acid; in fact, owing to the high molecular weight of glycogen, a vastly greater volume of such a solution would be required.

IRRITABILITY AND ANESTHESIA IN PLANTS¹

BY WILDER D. BANCROFT AND J. E. RUTZLER, JR.¹

That mysterious thing called protoplasm is acknowledged to be the essential component of living things, being directly associated with the properties by which man is able to distinguish the living state, namely respiration, growth, reproduction, movement, and irritability. Proteins are integral constituents of protoplasm. Provided one does not adhere to an exclusively rigid set of conditions by which to characterize the state of anesthesia, all living things, then, can be anesthetized. According to no less an authority than Claude Bernard,² "the action of anesthetics is very general. They react not only with animals but also with plants."

Many cases illustrate this: i.e., mammals by ether, fish by urethane, yeast cells by alcohol, frogs by cold, trypanosomes by arsenic, various bacteria by chloroform, various plants by ethylene, etc.

A natural consequence of these facts is the strong suspicion that the reversible coagulation theory of anesthesia should be applicable to plants. One finds further assurance that this is the case in the words of Bose:³ "In surveying the response of living tissues we find that there is hardly any phenomenon of irritability observed in the animal which is not also found in the plant. The various manifestations of irritability in the plant have been shown to be identical with those in the animal. The study of the responsive reactions in plants must, therefore, be regarded as of fundamental importance in the elucidation of various phenomena relating to the irritability of living tissues."

Bose and Das,⁴ partly because of the conduction of impulses by *Mimosa pudica*, take the stand that the plant contains conducting or nervous tissue. Many plant physiologists oppose this idea rather violently, however. Whether or not plant tissue is so highly differentiated as to contain a nervous system makes little difference; the important thing appears to be that there is some sort of parallelism between the plant and animal kingdoms as regards the conduction of a stimulus. In his earlier work Bose⁵ found that *Mimosa* is excited upon the application of electric current in a manner which is entirely comparable to animal nerves. For instance, in both the muscle of a frog and in *Mimosa* fatigue under continuous stimulation is found to take place. Likewise there is a preliminary staircase response under successive stimulations which is followed by fatigue both in the frog muscle and in *Mimosa*. The graphic record of these responses is strikingly similar in the two cases.

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¹ Eli Lilly Research Fellow.

² "Leçons sur les anesthésiques et sur l'asphyxie" (1875).

³ "Researches on Irritability of Plants," 360 (1913).

⁴ Proc. Roy. Soc., 98B, 290 (1925).

⁵ "Researches on Irritability of Plants" (1913).

Herbert¹ even goes on record to the effect that there is no anesthesia in plants, basing his case on work done with the sensitive plant (*Mimosa pudica*). The grounds upon which he bases this idea are not clear; in the end the matter is evidently only one of terminology.

Using wild sorrel leaves and hyacinths Lumière and Couturier² claim to have produced anaphylaxis in plants, the antigen being horse serum. The anaphylactic response was evidenced by the death of the plant several days after the shocking dose of antigen was injected. Onion bulbs were likewise subject to what Lumière designates as anaphylaxis; here monkey serum was the antigen. Picado³ claims to have demonstrated the formation of antibodies to foreign proteins injected into cacti of the genus *Opuntia*. These views are opposed by Lougo⁴ who concludes that one can only have anaphylaxis in animals. (All it what you may, it is doubtless true that there is a response in certain previously sensitized plants to the injection of foreign proteins. It seems better at the present time to steer clear of the conception that anaphylaxis occurs in plants; the important point nevertheless is that both plants and animals respond, in their own way of course, to foreign proteins.

These things of course do not make a plant into an animal, but they do provide a rational basis for the assumption that the reversible coagulation theory of anesthesia should apply in principle, if not in detail, to plants.

Changes in turgor are, according to Mathews,⁵ responsible for movements in many plants, an example of this principle being the sensitive plant. MacDougal⁶ extended this idea to cover the growth of plants. Supporting his theory that plant protoplasm is a colloidal system of a pentosan, albumin, and soap he found that compounds which promote growth in plants increased the hydration capacity of such a mixture. In another paper MacDougal⁷ reports that calcium chloride solutions induce the maximum swelling of gelatin in 0.001 M concentration, whereas the hydration is cut down as the concentration of calcium chloride increases or decreases from this optimum.

In animals the cycle, normality, irritability, anesthesia, irritability, normality, has been observed repeatedly with a variety of anesthetics.⁸ There is much that indicates a similar response to anesthetics by a variety of plants. Lepeschkin⁹ found that the fading of the corolla of *Chicorium Intybus* under the influence of narcotics cannot be brought about by the formation of a poisonous substance in the cells. Low concentrations of narcotics prolonged the life, but higher concentrations accelerated the fading. The significance of this work would have been greater had the author correlated his facts better, for he found that protein denaturation is the cause of heat fading. Yet he

¹ Philippine Agriculturist, 11, 141 (1922).

² Compt. rend., 172, 1313 (1921).

³ Ann. inst. Pasteur, 35, 893 (1921).

⁴ Lougo and Cesaris-Demel: Chem. Abs., 10, 3294 (1925).

⁵ "Physiological Chemistry," 205 (1915).

⁶ Am. J. Bot., 8, 296 (1921).

⁷ Proc. Am. Phil. Soc., 60, 15 (1921).

⁸ Bancroft and Rutzler. J Phys Chem., 35, 1185 (1931).

⁹ Am. J. Bot., 16, 324 (1929).

does not mention the relation between this and the fading due to narcotics. Nichols¹ is more helpful. He applied chloroform to the wall of an internode of *Nitella* by means of a capillary pipette. As the point of application of the anesthetic an area of non-motile protoplasm was produced. This area of non-motile protoplasm ultimately resumed movement; thus the process appears to be a reversible one. Nichols considers that the effect of the chloroform is due to gelation; this provides us with a definite case of reversible coagulation in plants accompanied by anesthesia of the part affected.

Magnesium sulphate is a well-known anesthetic and might therefore be expected to act as such for plants. Canals² found that above five parts of magnesium sulphate per 10,000 parts of water the compound is toxic to the roots and stems of plants. Magnesium sulphate in less concentrated solutions has a stimulating ("favorable") action on the stems. Distilled water can be used as a local anesthetic,³ and Heilbrunn⁴ found that the protoplasm of the eggs of sea-urchins can be coagulated by distilled water. It is not surprising to find, therefore, that distilled water exerts a toxic action on plants.⁵ It was found that the action is not always stopped by adding salts; it depends upon what salts are added and upon their concentration. Heat and mechanical coagulation of proteins are not new phenomena. Herbert⁶ recognized that heat produces an excitatory fall in *Mimosa* resembling in all respects that produced by mechanical shock. Wallace,⁷ in a recent paper, made a quantitative study of the effect of temperature upon the sensitive plant by measuring the angle of movement of the branches at different temperatures. Some of his data follow. The data show three very interesting things; the sensitivity of *Mimosa* is lost at 60°, which is about the coagulation temperature of albumin; coagulation by cold is shown at 12.5°, a temperature at which there are no ice crystals present, and the temperature of maximum activity is approximately that of the animal body. Egg albumin may be coagulated

TABLE I

Temperature °C.	Degrees— Angle of Movement	Temperature °C.	Degrees Angle of Movement
12.5	0.00	40.0	81.3
14.0	4.1	45.0	75.7
16.0	10.3	47.5	56.5
20.0	32.3	50.0	38.1
25.0	54.6	55.0	21.6
30.0	69.3	60.0	±0.00
35.0	75.7		

¹ Bull. Torrey Bot. Club, 57, 153 (1930).

² Chem. Abs., 15, 2654 (1921).

³ Wyeth: N. Y. Med. J., 1906, 6.

⁴ Biol. Bull., 39, 307 (1920); Exp. Zool., 30, 211 (1920).

⁵ Lesage: Chim. et Ind., 15, 281 (1926).

⁶ Philippine Agriculturist, 11, 141 (1922).

⁷ Am. J. Bot., 18, 293 (1931).

reversibly by cooling,¹ and frogs may be made completely insensitive to external influences² by putting them in water at nearly 0°. Likewise they undergo a heat narcosis in very warm water. The parallelism between plants and animals in their responses to heat and cold coagulation seems most striking.

Tadokoro³ made a very interesting study of the colloid chemistry of plant plasma using the press juice from macerated wheat sprouts. The chlorides of aluminum, magnesium, calcium, strontium and barium were found to flocculate the colloidal suspension, the order of the cations being: $\text{Al} > \text{Ba} > \text{Sr} > \text{Ca} > \text{Mg}$. Certain monovalent cations peptized the suspension in the following order: $\text{K} > \text{NH}_4 > \text{Na}$. So far as these series go, they are the same as the series for egg albumin. How much the peptizing action of KCl , NH_4Cl and NaCl is due to the chloride ion does not show up. It seems certain that at some point in the series the peptizing action of the anion must overbalance the coagulating effect of the cation. Salt concentrations which poison wheat were found to cause colloidal changes in the press juice. Using the following salt pairs, $\text{NaCl}-\text{CaCl}_2$; $\text{KCl}-\text{CaCl}_2$; $\text{KCl}-\text{MgSO}_4$; $\text{K}_2\text{SO}_4-\text{MgCl}_2$; and $\text{CaCl}_2-\text{MgCl}_2$, Tadokoro showed that in each pair there was a colloidal antagonism. These experiments were paralleled by pot culture experiments which showed a corresponding effects of the same pairs on the growth of the wheat plant. Peptone coagulated the press juice whereas glucose peptized it. Glucose and CaCl_2 were then found to be mutually antagonistic as well as KCl and peptone. The conclusion was drawn that the essential cause of the antagonism lies in the maintenance of a certain optimum degree of colloidal dispersion. For example, sodium chloride tends to peptize and calcium chloride tends to coagulate, so that the proper balance between them maintains the optimum dispersion.

Loew⁴ found that potassium iodide, manganous sulphate, ferrous sulphate, and sodium nitrate all stimulate the growth of plants. This brings up the question of irritability or stimulation to which the theory of reversible coagulation in living tissue appears to be, in part at least, applicable. Bose⁵ found that on bringing a highly excited sensitive plant into a dark room its excitability disappeared. The *Mimosa* is fully sensitive at night, however, and in the dark room its sensitivity slowly reappeared. Thus it appears that the colloidal equilibria of the plant are very delicately balanced and easily upset. Absorption of water also depressed the excitability of *Mimosa*. It was then found that glycerin restored the excitability of the plant; according to Bose this feat was not necessarily accomplished by the removal of water from the plant by the glycerol. Even though the continuous application of glycerol did not later cause a decline in the sensitivity, it does not seem improbable that it acts as a peptizing agent for the colloids of *Mimosa*. Sodium nitrate hastens

¹ Bancroft and Rutzler: *J. Phys. Chem.*, **35**, 151 (1931).

² Claude Bernard: "Leçons sur les anesthésiques et sur l'asphyxie" (1875).

³ *Chem. Abs.*, **14**, 960 (1920).

⁴ *Chem. Ztg.*, **48**, 391 (1924).

⁵ "Researches on Irritability of Plants," 87 (1913).

the sprouting of dormant potato tubers.¹ This effect may be due to two factors neither of which seems to be excluded. The nitrate ion, being a peptizing agent for proteins, probably acts in that manner with the potato tubers. Also, plants can utilize nitrates in their food economy. In a later paper Denny² reported that ethylene chlorhydrin and sodium thiocyanate hasten the sprouting. Employing different concentrations of sodium thiocyanate the amount of germination was greatest with a 1/2% solution, being less with a 1% solution, and still less with a 2% solution. In comparison with ethylene chlorhydrin, sodium thiocyanate may be a more powerful germinating agent. The temperature at which the tubers were soaked was less important when using sodium thiocyanate, and with this compound higher temperatures could be used. For breaking the dormant period ammonium thiocyanate was found³ to be better than sodium thiocyanate. There was not much margin between the stimulative and toxic dosages in the cases of sodium and potassium thiocyanate. Denny also found that ethyl iodide and o-tolylthiourea hastened sprouting. It requires no stretch of the imagination to class these compounds as peptizing agents. Analysis of the tissues⁴ from potato tubers after treatment showed no difference between the moisture content of treated and untreated tissue. This implies that the ethylene chlorhydrin and sodium thiocyanate did not act through a change in bound water in this particular case. In another paper⁵ Denny and Stanton report that the dormant period of the lilac can be broken by treatment with ether or chloroform. Ethylene in a concentration of one part per 100 when applied for 3 days to the flowering almond caused early blooming. They say that treatment with ethylene chlorhydrin or with ethylene dichloride breaks the rest of many plants and causes the flowers to come out.

It would appear at first sight that from the point of view of reversible coagulation the theory of the breaking of the rest period of plants is in a somewhat garbled state since both peptizing agents and coagulating agents accomplish the result. It is not entirely foreign to the realm of reason that one coagulating agent acting upon the same substrate may relieve the action of another coagulating agent. For instance, Stark⁶ obtained good results when combating neuritis with sodium iodide, an excellent peptizing agent. However, coagulating agents such as arsphenamine are also used⁷ with the production of beneficial effects. If we make the plausible assumption that in the dormant state the plant colloids are in an agglomerated condition the whole thing straightens out nicely. The action of certain coagulating agents would then be to displace the things causing the dormancy, the necessary condition being that the displacing compound shall be more strongly adsorbed, but not so

¹ *Am. J. Bot.*, 13, 122 (1926).

² *Am. J. Bot.*, 15, 395 (1928).

³ Denny: *Am. J. Bot.*, 13, 386 (1926).

⁴ *Am. J. Bot.*, 16, 326 (1924).

⁵ *Am. J. Bot.*, 15, 327 (1928).

⁶ *Neb. State Med. J.*, Norfolk, 9, 1 (1924).

⁷ Dutton: "Intravenous Therapy," 398 (1925).

good a coagulating agent, as the things that it displaces. In thus decreasing the degree of agglomeration of the colloids of the dormant plant a stage of stimulation may be reached. Quite obviously not all coagulating agents can fulfill these criteria and cause stimulation. The action of such things as sodium nitrate and sodium thiocyanate is clearly one of peptization thus taking the plant through the stage of stimulation which is observed just before anesthesia and during the recovery period.

Bose¹ studied the effect of various things on *Desmodium*. A dilute alcohol solution acted as a stimulant, whereas the action of a concentrated alcohol solution was that of a depressant. Ether first stimulated the plant; this was followed in succession by depression and narcosis. As would be expected chloroform acted qualitatively like ether. Carbon dioxide acted as a depressant, falling in line with the fact that it acts as an anesthetic² for rabbits and dogs. Acid and alkali acted the same way that they do on the mammalian heart.

The literature reveals several interesting things about *Mimosa*. Bose³ says: "Taking a plant in a subtonic condition, then, we may expect that any application of stimulus will increase its excitability, a fact which will find expression in a growing amplitude of response. This enhancement of excitability will reach a limit at which the plant will be in an optimum condition. After reaching this climax there may be a reversal, with a decline of excitability, a state of things which we associate with fatigue." On page 3 he says: "The most prominent motile organ in *Mimosa* consists of a mass of tissue known as the pulvinus, at the joint or articulation of the primary leaf stalk. The swollen mass on the lower side of this organ is very conspicuous. Under excitation the parenchyma, in this more effective lower half, undergoes 'contraction,' in consequence of which there is a fall of the leaf. This sudden movement constitutes the mechanical response of the leaf to the impinging stimulus, just as the contractile movement of a muscle in similar circumstances forms its characteristic mechanical response." Blackman and Paine⁴ report that an excised pulvinus of *Mimosa* when placed in warm water with its internal tissues freely exposed, exhibits, on stimulation, repeated contractions during many hours. This indicates that the loss of turgor in the cells of the lower half of the pulvinus, which is associated with contraction, cannot be explained by a sudden increase of permeability of the tissues allowing a rapid exosmosis of osmotic substances.

Turning again to the work of Bose⁵ we find that ozone stimulates *Mimosa*. Carbon dioxide depresses the excitability of the plant, and fresh air restores the plant to normal. Here we have a case of reversible coagulation as the cause of anesthesia. Dilute alcohol vapor sometimes causes a transient increase in excitability; continued application of the vapor causes a depression.

¹ "Researches on Irritability of Plants," 334 (1913).

² Leake and Waters Anesthesia and Analgesia, 8, 17 (1929).

³ "Researches on Irritability of Plants," 70 (1913).

⁴ Ann Bot, 32, 69 (1918)

⁵ "Researches on Irritability of Plants," 89 (1913).

Ether depresses the excitability of the plant after an initial short-lived stimulation, producing anesthesia. Carbon disulphide acts in a manner similar to ether. Chloroform acts as a very strong narcotic; the plant exhibits a long recovery period after anesthetization with chloroform.

Wallace¹ made the interesting observation that in the presence of alcohol-free ether vapor, ". . . a completely anesthetized *Mimosa* plant is practically identical in appearance with a normal plant for the same time of day. If the plant is exposed to ether during the day, when the leaflets are entirely expanded and the petioles up, the plant remains in exactly this position after anesthetization." He found that he could not anesthetize *Mimosa* in the position that it assumes after stimulation. He found that the leaflets do close up upon anesthetizing with chloroform. "I have obtained closure of some leaflets in concentrations of chloroform as low as 0.1%. I might also say that with the exception of ether, nitrous oxide, ethylene, and acetylene, all of the compounds which I have tested induce a chemonastic response similar to that with chloroform.

"*Mimosa* plants will retain their sensitivity from one to four hours in approximately 100% nitrous oxide and ethylene." The fact that nitrous oxide and acetylene do not behave as narcotics for *Mimosa* under ordinary conditions falls nicely into line with experiments showing that nitrous oxide does not flocculate yeast cultures, and is supposed, along with acetylene to act as an indirect narcotic,² having no direct coagulating effect.

Wallace also found that ethyl alcohol is very toxic to *Mimosa*, a concentration of 3% being lethal to the leaflets within ten minutes. "The most interesting characteristic of this alcohol is, however, that the sensitivity of the pulvini increases with increased vapor concentration, apparently to the lethal concentration." He noticed a periodic fall and rise of the petioles under the influence of alcohol.

Experimental Study

1. *Mimosa pudica*, the sensitive plant.

Professor Knudson of the College of Agriculture was good enough to grow a number of *Mimosa* plants for us. We thank him for this and for valuable suggestions in regard to the experiments.

The leaves of a branch were caused to fold up and the petioles fall by means of a mechanical stimulus. The first signs of recovery were manifested in 15 minutes, and the plant was fully recovered in 31 minutes. The temperature of the greenhouse was 21°. A second plant was sprayed with a 5% solution of sodium salicylate. Some of the leaves closed during the spraying process; it was fully recovered in 13 minutes. The plant was sprayed again 32 minutes after the first spraying, and some of the solution was used to water its roots. The sodium salicylate did not appear to be absorbed very readily by the leaves of the plant. A third plant was sprayed with a 1% solution of sodium amytal in order to find out whether or not this anesthetic affects

¹ Am. J. Bot., 18, 221 (1931).

² Bancroft and Richter: J. Phys. Chem., 35, 254 (1931).

plants. Thirty-two minutes later the plant was again sprayed with the sodium amytal solution. The sodium amytal solution appeared to be more rapidly absorbed than any of the solutions that were used, because under identical conditions the leaves appeared to dry most rapidly. Sodium amytal is apparently not an effective anesthetic for *Mimosa*. A fourth plant was sprayed with a 5% solution of sodium citrate that was just acid to litmus. The plant recovered from the fall due to the mechanical stimulation of the impinging droplets pretty well in nine minutes. It was sprayed again with the same solution after 30 minutes; and some of the solution was used to water it. A fifth plant was sprayed with a 5% solution of sodium thiocyanate; the process was repeated and the plant watered with the solution 29 minutes later. The time of recovery is not important in these experiments because it depends on the degree of sunlight and the experiments were not done simultaneously.

The sensitivity of the treated plants to mechanical stimulus was then tested. The plant that was treated with sodium salicylate was more sensitive in some spots than in others, probably because of an unequal absorption of the salt; white spots of sodium salicylate could be seen on the leaves. This plant seemed to be quite a little less sensitive than an untreated plant, the leaves did not fold up all the way upon stimulating them. The plant that was subjected to the sodium amytal solution responded to stimulation like a normal plant. The plant that was sprayed and watered with sodium thiocyanate was by far the least sensitive to mechanical stimulation; even comparatively powerful blows elicited only a faint folding response on the part of the leaves; and the petioles dropped only slightly. The plant that was treated with sodium citrate was more sensitive than any of the other treated plants.

A test was made upon the rate of recovery from a mechanical stimulus sufficiently powerful to drop the primary petioles and cause the leaves to fold. The plant treated with sodium thiocyanate was not included because so violent a shock was necessary to cause a dropping of the petioles that the plant was actually anesthetized, although it turned out to be as slow as the sodium salicylate plant in recovering. Thirty-nine minutes after the start of the experiment the treated plants had recovered in the following order from most to least: sodium amytal > sodium citrate > control > sodium salicylate. At the same time the order of sensitivity of the plants as nearly as could be determined was: sodium thiocyanate < sodium salicylate < control > sodium citrate > sodium amytal. At first sight it appears that there is no rhyme or reason to these results, but closer inspection reveals much. The symptomatology of coagulation and of over-peptization may quite reasonably be the same. Consider a collodion bag, closed at both ends and filled to 85% of its capacity with egg white containing 10% of ammonium sulphate. Now, if one immerses this contraption in distilled water it will fill to the breaking point by osmosis and become more resistant to bending than it formerly was, it being against the rules to break the bag. Likewise, if one immerses the system in boiling water the albumin will coagulate to a hard mass thus also making the bag unbendable. Qualitatively the same outward result, then, has been achieved both by coagulation and peptization. In the sensitivity series we have sodium

thiocyanate and sodium salicylate acting as peptizing agents; while sodium amytal and sodium citrate are acting as coagulating agents. As one would expect, sodium amytal is a better coagulating agent than sodium citrate. At the same time they all produce the same symptoms. The recovery series can be taken as a comparison of the state of excitability of the individual plants. The least recovered plants, those treated with sodium salicylate and sodium thiocyanate, were quite obviously in the state of least excitement; those treated with sodium amytal and sodium citrate were in the most excited condition. If these data are regarded as accurate enough, the sensitive plant falls into line beautifully with the reversible coagulation theory of anesthesia.

From the behavior of the plants treated with sodium thiocyanate and sodium salicylate it would appear that the peptization phenomenon in these cases is intimately concerned with the imbibition of water. Cytolysis is, therefore, probably the cause of death by toxic doses of sodium thiocyanate.

A 16-liter bell jar was inverted over a healthy sensitive plant, a wad of cotton on which was poured five cc. of ether, containing about 0.3% of alcohol as impurity, was placed beside the pot. The bell jar was then sealed to a glass plate. After 38 minutes the plant did not appear to be anesthetized. When removed at this time neither the pulvinus nor the leaves had lost their sensitivity; on the contrary, they appeared to be more sensitive than they normally would be. The plant was probably in the stage of excitation. The process was repeated using another plant and three times the amount of ether was used. This plant was not completely anesthetized in one hour 36 minutes. The sensitivity of the leaves to mechanical stimuli was gone for they did not close upon being touched; but the pulvinus was extremely sensitive, because the petioles fell upon very mild mechanical stimulation. This is in accord with the finding of Wallace¹ that at higher temperatures the pulvini often remained sensitive after the leaflets and part of the primary petiole were entirely dead and dried up. At the same time that this plant was confined to the bell jar there stood beside it a plant that had been treated previously by twice spraying it with a 5% sodium thiocyanate solution. When removed from the anesthetizing chamber after one hour 36 minutes neither the pulvinus nor the leaves were sensitive to mechanical stimulation; the leaves were not closed and the petioles did not fall. That this is truly a case of antagonism between ether, a coagulating agent, and sodium thiocyanate, a peptizing agent, will be more evident in the light of the experiments that will be described next.

Five cc. of ether were poured on the soil in which a potted sensitive plant was growing without touching the plant. One leaf closed up and the petiole fell immediately. Complete anesthesia ensued in four minutes; both the leaves and the petioles were affected so that the plant was completely folded up. The sensitivity of the pulvini began to return several times as was evidenced by an extremely slight rise of the petioles, but each time they fell again. After one hour the pulvini were slightly sensitive and the petioles had risen

¹ Am. J. Bot., 18, 293 (1931).

a few millimeters; the leaves had not opened at all. At the same time a plant that had been sprayed twice with a 5% solution of sodium thiocyanate, and had stood for about 30 minutes after the second spraying, was treated by pouring five cc. of ether on the soil, being careful not to touch the plant. After six minutes the anesthetic had a slight effect; the leaves were not affected at all so far as one could see, for they did not close. In the case of the control plant the petioles dropped until they were supported by the edge of the pot; whereas in the case of the plant treated with sodium thiocyanate the petioles dropped about one quarter as far and were fully erect again 17 minutes after they fell.

Another plant was anesthetized by pouring five cc. of ether on the soil. It took five and one-half minutes for the ether to take effect, at which time the petioles fell and the leaves closed. After 40 minutes the pulvini were slightly sensitive as evidenced by a few millimeters rise of the petioles and by their dropping again when touched. Five cc. of ether was carefully poured on the soil of still another plant at the same time that the above plant was so treated. The leaves closed and the petioles fell in two and one-half minutes. A minute and one-half later the plant was sprayed with a 5% solution of sodium thiocyanate. Eighteen minutes later the leaves were partly open; the petioles were partly raised; and the pulvini were quite sensitive.

Thus it has been shown that it is possible to reduce markedly the narcotic action of the coagulating agent ether upon the sensitive plant by employing the well-known and efficacious peptizing agent, sodium thiocyanate. Likewise, as the theory demands, sodium thiocyanate aids materially in the recovery of *Mimosa* from anesthesia.

2. *Lycopersicon lycopersicum*, the tomato plant.

‘rocker’ reports that “a vigorously growing tomato plant will have its leaves turned back to a noticeable degree by concentrations of illuminating gas (with 9 to 10% illuminants) as low as one part of gas in 100,000 to 200,000 of atmosphere.” Ethylene was found to be the constituent mainly responsible for the effect. “The response of the tomato plant to gas is a growth response. . . .”

A paper by ‘rocker and Knight² contains the following summary: “The flowers of the carnation are extremely sensitive to traces of illuminating gas in the air.

“With the Boston Market and Pink Lawson three days exposure to 1 part in 40,000 kills the young buds and prevents the opening of those already showing the petals. The buds of medium age are considerably more resistant.

“In the same varieties 1 part in 80,000 causes the closing of the open flowers upon twelve hours’ exposure.

“This injury takes place directly on the bud or flower exposed and not indirectly through absorption by the roots.

“The ‘sleep’ of the carnation is probably often caused by traces of illuminating gas in the air.

¹ Florists Exchange and Horticultural Trade World, 70, 15, 54 (1929).

² Bot. Gaz., 46, 259 (1908).

"Ethylene is even more fatal to the flowers of the carnation.

"Twelve hours exposure to 1 part in 2,000,000 causes the closing of flowers already open."

Ethylene also causes the leaves of *Mimosa* to drop, which means that in that case also it acts as an anesthetic.

With these things in mind attempts were made to apply the reversible coagulation theory of anesthesia to tomato plants after they had been acted upon by ether in one case, and by ethylene in another case. Two young, recently potted, tomato plants were placed in a 14 liter bell jar which was maintained at 26°, to within one degree by means of an electric light above the jar. Ten cc. of ether, in an evaporating dish, were placed in the bell jar which was then sealed to a glass plate. The experiment was started at 9:35 a.m. At 5 p.m. the only noticeable effect of the ether on the plant was a slight drooping of the leaves and petioles. Ten cc. more ether was placed in the evaporating dish at this time.

At 12:15 a.m. both plants were drooping and limp, so they were removed from the anesthetizing chamber. One plant appeared to be in a little poorer condition than the other; this plant was sprayed immediately with a 5% solution of sodium thiocyanate, and watered with 15 cc. of the solution at the same time. The other plant was sprayed with tap water and watered with 15 cc. of the same. The plants gave off quite a little water while in the bell jar. Forty-five minutes after the plants were watered and sprayed they were in bad shape. At this time the plant treated with water did not appear to droop so much and was not so limp as the one treated with sodium thiocyanate. It was noticed that the leaves of the control plant tended to curl up laterally; whereas the leaves of the plant treated with sodium thiocyanate exhibited no such tendency. Also, the control plant appeared to be more withered than the other. An hour and one-half after the plants were removed from the anesthetizing chamber and treated, the control plant appeared to be in much worse general condition than the one treated with sodium thiocyanate.

At 10:00 a.m., or a little more than 24 hours after the start of the experiment the leaves of the control plant were shrivelled, dry, and crisp; the leaves of the other plant were shrivelled but neither dry nor crisp. This same condition of affairs persisted for at least three more days. The stem and petioles of the plant treated with sodium thiocyanate apparently shrank a great deal. This observation gave rise to the suggestion that perhaps the tomato plant is impermeable to the thiocyanate ion. A qualitative test for the ion was strongly positive indicating that the plant is probably permeable to it.

The next experiment was performed using ethylene as the anesthetic. Two tomato plants were placed under a 16 liter bell jar containing 25 cc. of ethylene at 6:00 p.m.; at 10:40 p.m. the plants did not seem to have responded to the anesthetic so the bell jar was aired out and 50 cc. of ethylene put into it. At 12:15 a.m. the primary petioles of both plants were curled up so that the far tips of the leaves touched the main stems. The appearance of the primary petioles was the same as described by Crocker¹ for all of the petioles of a

¹ Florists Exchange and Horticultural Trade World, 70, 15, 54 (1929)

vigorous young plant. One plant appeared to be a little bit more affected by the ethylene than the other.

At 12:15 a.m. the plant that was in the worse condition was sprayed with a 5% solution of sodium thiocyanate and watered with 15 cc. of the solution. The other plant was treated in exactly the same manner, only tap water was used instead of sodium thiocyanate solution. Forty-five minutes later the primary petioles and leaves of the control plant were in exactly the same position as they were at the time the plant was sprayed. The leaves and petioles were perhaps a little more stiff and resistant to touch than those of an untreated plant. The plant that was treated with sodium thiocyanate presented an entirely different picture. The leaves and petioles were drooping and limp. The leaves of the control plant were erect on the petioles and the petioles were bowed.

The control plant still showed very markedly the effect of the anesthetic 24 hours after it was removed from the atmosphere containing ethylene. At this time the plant treated with sodium thiocyanate was in very bad condition; all of the petioles had fallen and the leaves were shrivelled, but they were not dry. Although the sodium thiocyanate was apparently toxic to the plant the leaves had not dried out even after three days. It was found in a later experiment that a 1% sodium thiocyanate solution is slightly toxic to the tomato plant.

It is important to note that the sodium thiocyanate acted upon the primary petioles, which were the ones most affected by ethylene, within 45 minutes; whereas, it took quite a few hours for the other petioles to respond to the sodium thiocyanate.

These experiments show that the death of the tomato plant from an overdose of ether is retarded by sodium thiocyanate, as it should be if the theory of reversible coagulation holds. Likewise, as one would predict, the stimulation brought about by ethylene is counteracted by sodium thiocyanate.

The effect of sodium thiocyanate may be produced in either or both of two ways. In the first place it may peptize the colloidal system of the plant directly. Since we know of no protein that is not peptized by sodium thiocyanate, it seems that this mechanism must always be a part of the picture. Also, the sodium thiocyanate may alter profoundly the capacity of the plant cells to imbibe water, in the direction of greater imbibition. This seems to be the preponderant action in the case of the sensitive plant. That sodium thiocyanate repeptizes the reversibly coagulated colloids of a plant affected by an anesthetic seems certain, and is as it should be. Here again it is important that the amount of coagulation induced by an anesthetic may be so small as to go unnoticed even under the ultramicroscope. The same thing holds for the peptization of the plants colloids *in vivo*.

The general conclusions supported by this paper are as follows:

1. In animals and plants the effect of an anesthetic upon the bio-colloids is that of reversible coagulation.

2. Ether, chloroform, alcohol, distilled water, magnesium sulphate, carbon dioxide, ethylene, heat, and cold have been found to anesthetize a variety of plants by various workers.

3. Plants respond in a manner similar to animals to electrical stimulation. Fatigue and various irritability phenomena occur alike in plants and animals.

4. Anesthetics produce in plants a preliminary stage of excitation, as they should.

5. Protein coagulation is the cause of the depression of plants by heat.

6. The term reversible gelation is applied by Nichols to the effect of chloroform on *Nitella*.

7. The colloids from macerated wheat sprouts are flocculated by many cations, the lyotropic series being the same as that for egg albumin. Likewise, there are ion antagonisms of the same sort as are found in animals.

8. The effects of sodium thiocyanate, sodium salicylate, sodium citrate, sodium amyral, and ether on plants are exactly as the theory of reversible coagulation demands; the first two peptize the bio-colloids, and the last three coagulate them.

9. Anesthesia of the sensitive plant by ether has been antagonized by sodium thiocyanate by employing the salt before the anesthetic and after the anesthetic.

10. Sodium thiocyanate causes *Mimosa* to become rigid, due probably to the imbibition of water by the colloids of the plant.

11. The death of the tomato plant as a result of over-exposure to ether is greatly retarded by sodium thiocyanate.

12. Sodium thiocyanate has, as one would expect, a more rapid action upon those leaves of the tomato plant that are most affected by ethylene.

13. The effect of ethylene upon the tomato plant appears to be counteracted by sodium thiocyanate.

14. The action of ethylene upon the tomato plant is one of stimulation which means in the terms of this theory partial coagulation of the bio-colloids. Sodium thiocyanate appears to reeptize these colloids.

15. Sodium thiocyanate breaks the dormant period of potato tubers. This is exactly what should happen if the colloids of the dormant tubers are somewhat agglomerated.

16. Claude Bernard's theory of anesthesia as developed in the laboratories of the senior author applies without modification to plants.

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COLLOIDAL PHENOMENA IN GALL STONES

BY HARRY B. WEISER AND GEORGE R. GRAY

The importance of colloidal behavior in the formation of gall stones and other concretions has been recognized for a long time. Thus Hippocrates and Galen, the fathers of medicine, attributed such formations to an accumulation of mucus which clung to the organ and served as a nucleus for the stone which subsequently formed. The first experimental evidence which indicated the rôle of colloids in concrement formation was obtained by A. von Heyde who dissolved out the crystalline material from urinary calculi and observed a residual framework. This was recognized quite clearly by Meckel von Helmbach in his book on Microgeologie published in 1856. Thus he writes: "Two basic factors underlie the formation of every true gall or urinary stone; first, the presence of an organic substance, mucus, in which there may be deposition of salts; second, a suitable urinary or gall fluid to serve as the mother liquor for these sediments. The decomposable organic substance, mucus, is unquestionably necessary, because urinary salts and gall substances of themselves can yield only crystalline, pulverulent or granular precipitates and never larger pieces. Stones are formed only when an organic binder is carried down too."

While the presence of an organic binder resulting from inflammatory processes has been definitely established as essential for the formation of certain types of gall and urinary stones,¹ it was demonstrated twenty years ago by Aschoff² and by Schade³ that both gall and urinary calculi may form under certain conditions without being accompanied by inflammation. In this paper the colloidal phenomena which are concerned with the formation of gall stones without coexisting inflammation will first be reviewed, after which gall stones formed during inflammation will be considered especially from the point of view of the mechanism of the formation of the concentric rings or layers in such stones.

Gall Stones formed without Inflammation

When stones are formed in the gall bladder without the coexistence of inflammation there is usually but a single stone composed largely of cholesterol. Such stones are called "pure cholesterol stones" although they usually

¹ Pfeiffer: 5th Cong. Int. Med., Wiesbaden (1886); Posner: Arch. klin. Med., 5, (1885); 16, (1889); Naunyn: "Klinik der Cholelithiasis," Leipzig (1892); Gilbert and Dominici: Compt. rend. soc. biol., 28, 1033 (1893); Moritz: 14th Cong. Int. Med., Wiesbaden (1896); Gilbert: Arch. gén. de Méd., 2, 257 (1898); Gilbert and Fournier: Presse Med., 7, 259 (1898); Mignot: Arch. gén. de Méd., 2, 129, 263 (1898); Schreiber: Virchow's Archiv, 153, 147 (1898); Cushing: Bull. Johns Hopkins Hosp., 10, 166 (1899).

² Aschoff and Baemeister: "Cholelithiasis" (1909); Kleinschmidt: "Die Harnsteine" (1911).

³ Münch. med. Wochenschr., Nos. 1 and 2 (1909); Kolloid-Z., 4, 175, 26 (1909); Kolloidchem. Beihefte, 1, 371 (1910); Alexander's "Colloid Chemistry," 2, 801 (1928); cf., also, Boysen: "Über die Struktur und Pathogenese der Gallensteine" (1909).

contain small amounts of alkali and calcium cholates, bile pigments, etc. Fig. 1 shows a cross section of a stone which is nearly pure cholesterol. The specimen is white, hence is free from bile pigments. A portion of the stone was found to be almost completely soluble in ether, which indicates the absence of alkali or calcium cholates.

A probable mechanism of the formation of such "pure cholesterol stones" was suggested by Ord¹ and was extended and formulated by Schade.² The

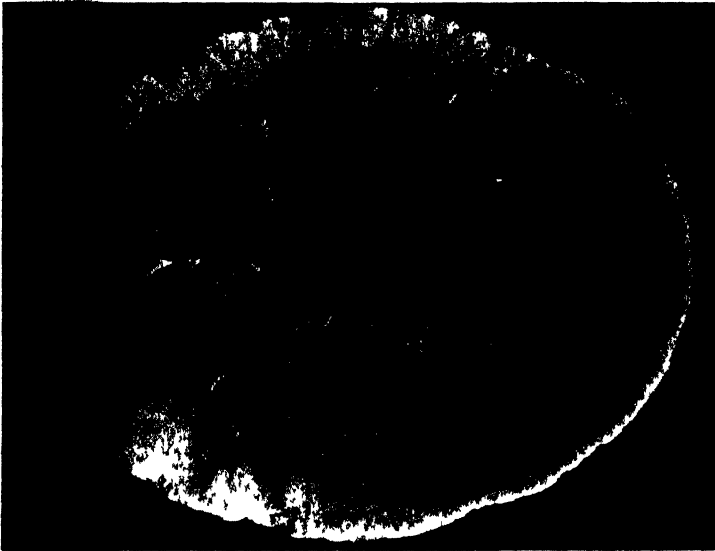


FIG. 1
Cross-Section of Pure Cholesterol Stone ($\times 6$)

latter demonstrated that when a 2 to 6 per cent solution of cholate is saturated with cholesterol at approximately 80° and the solution cooled rapidly, a cloud of formless white flocs of cholesterol separates out; if the cooling is quite slow relatively large crystals are obtained. On the other hand, if the hot cholate-cholesterol solution is shaken with a drop of olive oil, gall fat, or petroleum oil, and then cooled, the cholesterol separates in myelin-like drops with the oil phase. These drops, which at first are transparent soft bodies, coalesce and undergo crystallization with the separation of fat, giving radially crystalline spheres of almost pure cholesterol.

A similar phenomenon apparently takes place in stasis of the bile. This secretion which at first possesses a slight alkaline reaction consists of a mixture of alkali cholates, bile pigments, cholesterolin, lecithin, fats and soaps, as well as electrolytes which are widely distributed in the body fluids. The cholesterol is colloiddally dispersed in the bile fluid by the protective action of the alkali cholates. Now in stasis of the bile, it has been demonstrated by the surgeon

¹ "The Influence of Colloids on Crystalline Form and Cohesion" (1879)

² *Kolloidchem. Beihefte*, 1, 375 (1910)

and pathologist that the amount of cholates diminishes gradually owing to resorption and autolysis, leaving a fluid which may be clear and almost cholate free. This gradual disappearance of cholates leaves a supersaturated solution of cholesterol which separates out.¹ But owing to the presence of fat and soaps in the fluid, the separation is in the form of drops which coalesce and subsequently crystallize initiating "pure cholesterol" gall stones.

From the above it would appear that stasis of the bile may in itself lead to the formation of gall stones especially if there is an impairment of liver function giving a bile deficient in cholates. It is probable that there are other contributing causes not associated with inflammation, which cause the separation of the cholesterol, in such a condition that stones result. Thus Thudichum² points out that a stagnant and infected bile will decompose giving an acid reaction. This would convert the cholates into insoluble cholic acids which would allow the cholesterol to precipitate. In this connection Wrada,³ Neilson and Meyer⁴ and Rous, McMaster and Drury⁵ found that bile taken from the gall bladder was slightly acid in a large proportion of cases while that taken from the hepatic duct was nearly always somewhat alkaline and usually markedly so. Whatever may be the cause of the normal change in pH in the gall bladder, it occurred to Petersen⁶ and Miller that gall stones might be formed by altering definitely the pH value of the bile. A distinctly acid bile was obtained in a number of experimental animals by grafting a pedicled flap of the stomach to the gall bladder wall and an alkaline bile by anastomosis of the gall bladder to a portion of the duodenum. The bile from gall bladders containing the duodenal transplants was regularly alkaline and was more viscid than normal bile; but no evidence of stone formation was observed in six months. The gall bladders having the gastric transplants always yielded an acid bile. No definite concretions were obtained but the gall bladder wall appeared reddened and congested, with cholesterol particles scattered over the entire surface. This condition is what is known clinically as "strawberry gall bladder," recognized as a forerunner of gall stones.

These observations of Petersen disprove the contention of Porges⁷ that free, unneutralized acid will not remain in contact with the mucous membrane of the gall bladder and the claim of Baemeister⁸ that the addition of small amounts of acid to the bile is unsuitable for bringing about the precipitation of cholesterol.

The hypothesis that the destruction of cholates by bacterial action contributes to the formation of gall stones as claimed by Exner and Heirovosky⁹ and Bondi and Hess¹⁰ on the basis of observations in vitro, is open to the

¹ Cf. Oliver: *J. Lab. Clin. Med.*, 8, 242 (1923).

² Virchow's Archiv, 156, 384 (1899).

³ *J. Physiol.*, 50, 114 (1915).

⁴ *J. Infect. Dis.*, 28, 511 (1921).

⁵ *J. Exp. Medicine*, 39, (1) 77; (2) 97; (3) 403 (1924).

⁶ Unpublished paper.

⁷ *Kolloid-Z.*, 5, 301 (1909).

⁸ *Munch. med. Wochenschr.*, Nos. 5, 6, and 7 (1908).

⁹ *Arch. klin. Chu.*, 86, 609, 643 (1908).

¹⁰ *Wiener klin. Wochenschr.*, 21, 271 (1908).

objection that even under the extreme conditions chosen by them, there still remained enough cholates to keep the cholesterol dispersed.¹

As a result of chemical-pathological investigations, Chauffard² observed that stone formation in the absence of inflammation is frequently accompanied by a pathological condition which results in an increase in the cholesterol content of the blood. In such cases the primary cause is a disturbance in the metabolism.

Since cholesterol is colloiddally dispersed in the bile, Porges³ attempts to explain the precipitation by assuming the coagulating action of substances on the sol. His suggestion follows from analogy with the behavior of hydrosols of lecithin and cholesterol toward electrolytes. The difficulty is that one must postulate the presence in the bile of an amount of coagulating electrolyte considerably larger than is likely to be present.

While all of the above factors may contribute to the formation of gall stones in the absence of inflammation, it seems probable that sufficiently marked stasis of the bile to allow of resorption of the cholates is adequate in itself to cause stone formation provided the conditions are favorable for the separation of the cholesterol in myelin-like drops which coalesce and subsequently undergo crystallization. This would seem to be particularly true if the bile were deficient in cholates or especially rich in cholesterol as a result of impaired liver function.

In addition to the pure cholesterol stones, pure pigment stones⁴ and pure calcium carbonate stones⁵ are formed under certain conditions without apparent inflammation. Peel⁶ analyzed some pigment stones of this kind and found them to be entirely free from cholesterol. They contained some free pigment but were largely calcium and copper bilirubinate. The following description of these stones indicates that like pure cholesterol stones, they are formed as a result of the dropwise separation of the insoluble calcium salts of the bile pigments: "They vary in size from that of a pin-head to a cherry seed and possess a fine granular surface such that they appear as if they were built up of several small globules. In a fresh condition they are somewhat hard; on drying, the hardness increases enormously. In cross section they appear uniform throughout. They show neither radial nor concentric layers nor a distinguishable central core."

The formation of the pure calcium pigment stones is probably favored by a disturbed metabolism which gives a bile relatively rich in pigments and in calcium and possibly copper salts.⁷ It is probably true that this condition alone without inflammation would not cause the calcium pigment to precipitate as a concrement unless the initial separation was in the form of drops.

¹ Porges: *Kolloid-Z.*, 5, 301 (1909).

² *Leçons sur le lithiase biliaire* (1914).

³ *Kolloid-Z.*, 5, 301 (1909); Porges and Neubauer: *Biochem. Z.*, 7, 152 (1907).

⁴ Boysen: *Über die Struktur und Pathogenese der Gallensteine* (1909; Bacmeister. *Ergebn. inn. Med.*, 11, 1 (1913)).

⁵ Halpert: *Arch. Path.*, 6, 630 (1928).

⁶ *Z. physiol. Chem.*, 167, 269 (1927).

⁷ Peel: *Z. physiol. Chem.*, 167, 274 (1927).

Gall Stones of Inflammatory Origin

An inflammation in the bile ducts or the gall bladder introduces into the bile irreversibly precipitating hydrophilic colloids such as serum albumin, globulin, and fibrin. If cholesterol or calcium bile pigments separate in the presence of such colloids, there is mutual adsorption with the result that the whole is united into a coherent mass giving what is sometimes termed a colloid-crystalline stone as distinct from the pure crystalline stone considered in the last section. Since gall stones are usually of inflammatory origin, it follows that colloid-crystalline stones are the type most commonly found in the gall bladder.

As has been pointed out, the formation of stones in the absence of inflammation is probably preceded by the separation of the stone-forming material in a drop-like form. A similar condition may obtain when inflammation is present but in the latter case dropwise separation is not essential since the colloidal material resulting from the inflammatory process may bind the mass firmly into a concrement. Indeed, Schade¹ produced stones artificially by allowing coagulation of fibrin to occur in solution in which freshly precipitated, sediment-like discrete crystals were suspended.

The importance of the colloidal binding material in concrement formation is shown by dissolving out first one constituent and then the other from a colloid-crystalline stone. If the crystalline material is removed with a suitable solvent, a firm coherent skeleton of colloidal matter remains which shows the details of structure of the stone. On the other hand, if the albuminous skeleton is dissolved out by antiformin, there is complete disintegration, nothing remaining but a slimy mass of minute crystals.²

Since the relative amounts of the various constituents which make up gall stones of inflammatory origin may vary widely, it is obvious that the composition, appearance, and physical properties of the stone will show almost infinite variation. In a stone in which there is a relatively small amount of hydrophilic colloid, the soft irregular masses consisting largely of cholesterol, undergo solution and recrystallization, ultimately giving a radial structure similar to that in the pure cholesterol stone. Adsorption of the bile pigments or their precipitation as the calcium salt³ stains the stone to a greater or lesser degree depending on the relative amount of pigment adsorbed or thrown down. If the amount of hydrophilic colloids present in the stone is relatively large, crystal growth is inhibited and the formation of long crystals is prevented. Since the hydrophilic colloids lose water and shrink with time, stones containing a large amount of such colloids may become sufficiently friable that they fall to pieces. In other cases the ageing may make radial rifts leading out from the center leaving an irregular hollow space which fills with liquid.

¹ Münch. med. Wochenschr., Nos. 1 and 2 (1909).

² Schade: Med. Klinik, No. 15 (1911).

³ The so-called calcium salt of the bile pigments is probably an adsorption complex of indefinite composition.

The most common form of gall stones are the colloid-cholesterol-calcium pigment stones that are characterized by the presence of concentric rings varying in color. Such concretions are termed layered stones or "common gall stones." In these stones the kernel and the surrounding ring structure are readily distinguished. The former consists largely of albumin, calcium, bilirubin and cholesterol without the presence of a definite ring structure while in the latter, there are a number of colored rings in crystals of cholesterol.

Gall stones of inflammatory origin seldom or never occur singly. In rare instances there may be but two or three stones but, as a rule, there is a much larger number, 100 or more in some cases. Because of the pressure of the gall bladder the stones are seldom spherical but are faceted and usually of widely varying shapes. In certain cases the shape may be more or less uniform. In a collection obtained from one gall bladder there were 50 stones each of which was an almost perfect pyramid with rounded edges.

The cause of the concentric rings in the common gall stones is usually attributed to layering as a result of variations in conditions which produce alternate layers of cholesterol and pigment. Schade¹ attributes the formation of a layered rather than a radiating structure to the effect of pressure which flattens out the crystals of cholesterol. This does not account for the beautiful regular formation of alternate dark and light rings. Moreover, in most stones of this type there is a distinct evidence of radiating structure and in many cases this is quite marked as shown in the photographs of the cross-section of stones shown in Figs. 2 and 3.

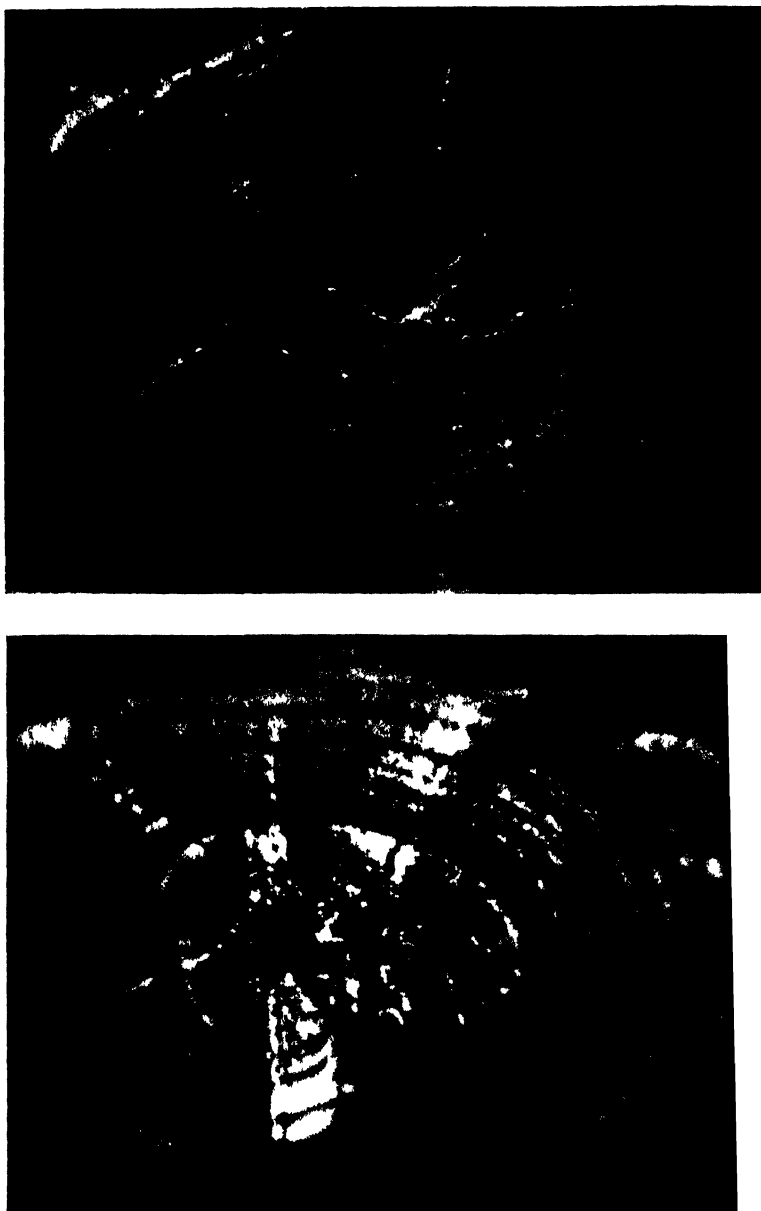
The appearance of the concentric rings suggests to the colloid chemist that they may originate as a result of the rhythmic banding first described by Liesegang as a result of his observations of the formation of rings of silver chromate when silver nitrate is allowed to diffuse into a gelatin jelly containing dichromate. The possibility that the rings in gall stones are not layers but rhythmic bands has been taken seriously by few people. Schade² dismisses the suggestion promptly by contending that for the formation of Liesegang rings as a primary process, the diffusion must take place in a jelly which has no points of resistance to diffusion. This, he points out, does not obtain in gall stones owing to the presence of crystalline cholesterol irregularly included in the framework of hydrophilic colloids. Moreover, he rules out the formation of Liesegang rings as a secondary process, since this would give very irregular lines, which do not occur.

Sweet³ takes the position that the concentric rings are due to the Liesegang phenomenon. He gets around the difficulty confronting Schade by postulating that the cholesterol forms a gel containing calcium into which the bile pigments such as bilirubin can diffuse giving rhythmic bands of calcium bilirubin just as silver nitrate diffuses into dichromate-gelatin jelly and gives rhythmic bands of silver chromate.

¹ Alexander's "Colloid Chemistry," 2, 817 (1928).

² Alexander's "Colloid Chemistry," 2, 830 (1928).

³ Colloid Symposium Annual, 8, 249 (1930).

**FIG 2**

Cross-Section of Natural Gall Stones showing both Ring and Radiating Structures. ($\times 10$).

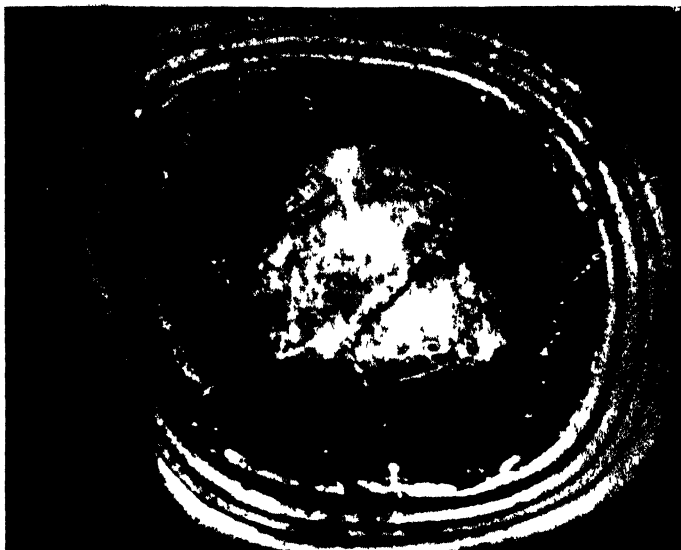


FIG. 3

Cross-Section of Natural Gall Stone ($\times 10$)

A consideration of the gall stone-bile system reveals, however, a marked difference between it and the gelatin-electrolyte system of Liesegang. In the first place, the gall stone is probably not a true jelly at any stage of its history, as assumed by Sweet. And if it did possess a true jelly structure like that of gelatin, rhythmic bands could not form as a result of diffusion since the bile pigments are in colloidal solution and so diffuse but little if at all. Schade's objection to the banding theory on the ground that rhythmic precipitation takes place only in a jelly, does not hold since the phenomenon manifests itself not only by diffusion into jellies but also into relatively non-uniform amorphous and crystalline masses. Accordingly, it is altogether possible that the precipitation of crystalline cholesterol in the presence of hydrophilic colloids will, under certain conditions, give a mass into which the colloidal pigment can diffuse and by interacting with lime or other calcium salt carried down with the cholesterol, precipitate colored rhythmic bands. In other words, the very fact that cholesterol is definitely crystalline may be the determining factor in producing rhythmic bands therein since a mass of small crystals would allow the interdiffusion of the colloidal bile pigments.

If this is a true statement of the case it should be possible to simulate the conditions sufficiently closely that rhythmic bands of calcium bile pigment will be formed in a mass of precipitated cholesterol containing lime. This has actually been accomplished as will be described in the following experiments.

Experimental

Rhythmic Bands of Ag_2CrO_4 in Cholesterol. To show that rhythmic banding will take place in a mass of cholesterol crystals, the following experiments were carried out: One gram of gelatin was dissolved in 100 cc. of water con-

taining 0.1 gram of K_2CrO_4 and heated to 70° . Into this solution was poured rapidly 25 cc. of a hot alcoholic solution of cholesterol containing 2 grams of the pure compound. The cholesterol precipitated immediately in the form of minute crystals. These were matted firmly and uniformly in the bottom of a test tube by centrifuging and the supernatant solution was poured off. The precipitate was then covered with an 8 percent solution of $AgNO_3$. Upon standing, the familiar rhythmic bands of Ag_2CrO_4 were formed.

The above procedure was repeated keeping all the factors constant except the amount of gelatin in the solution into which the alcoholic cholesterol was poured. The gelatin solutions contained 0.75, 0.50, 0.25 and 0.05 gram, respectively, in 100 cc. In every case rhythmic bands were formed. With decreasing amounts of gelatin the bands were broader, less distinct and further apart. When no gelatin was used, bands were not formed, but crystals of Ag_2CrO_4 were scattered irregularly throughout the mass.

Portions of the precipitate thrown down in the presence of a small amount of gelatin when placed on a watch glass and surrounded with silver nitrate gave rhythmic rings similar to those obtained by diffusion in gelatin.

Since bands of silver chromate in cholesterol are formed in the presence of such minute amounts of gelatin, it is obvious that the rhythmic precipitation is not taking place in a gelatin jelly. The gelatin merely serves to inhibit the growth of the cholesterol crystals and so to give a mass of minute crystals in which the diffusion phenomenon can take place under such conditions that rhythmic bands or rings result.

Rhythmic Bands of Calcium Bile Pigment in Cholesterol. Solutions of bile pigment were prepared in the following way. Twenty-five grams of finely powdered human gall stones were extracted in a Soxhlet tube with 200 cc. of ether for two days to remove cholesterol and fat. The residue was dried, washed with hot water, then with 10 percent acetic acid and finally with water. This residue was dried and ground in small portions with 2 N NaOH and the filtered solution was used in the experiments. When a portion of the highly colored solution was subjected to dialysis in a cellophane bag, the color did not diffuse showing that it was in the colloidal state just as it is in the bile fluid. The addition of a dilute solution of calcium nitrate did not result in immediate precipitation of the calcium-pigment complex but upon standing, a precipitate settled out which varied in color from reddish brown to olive green, depending upon the degree of oxidation of the pigment.

Although the colloidal bile pigments do not diffuse through gelatinous membranes, it seemed not unlikely, in the light of the above experiments with Ag_2CrO_4 , that a suitably precipitated mass of cholesterol crystals containing calcium would serve as a medium into which the colloidal pigments would diffuse to form rhythmic bands, thus simulating the process which probably takes place in nature. This hypothesis was confirmed by the following method of procedure: Into 50 cc. of a solution containing 0.5 gram of gelatin and 0.5 gram of calcium nitrate at 70° was poured 12.5 cc. of a hot alcoholic solution of cholesterol containing 1 gram of the pure compound. After prolonged centrifuging the supernatant solution was removed from the mass of precipitate and

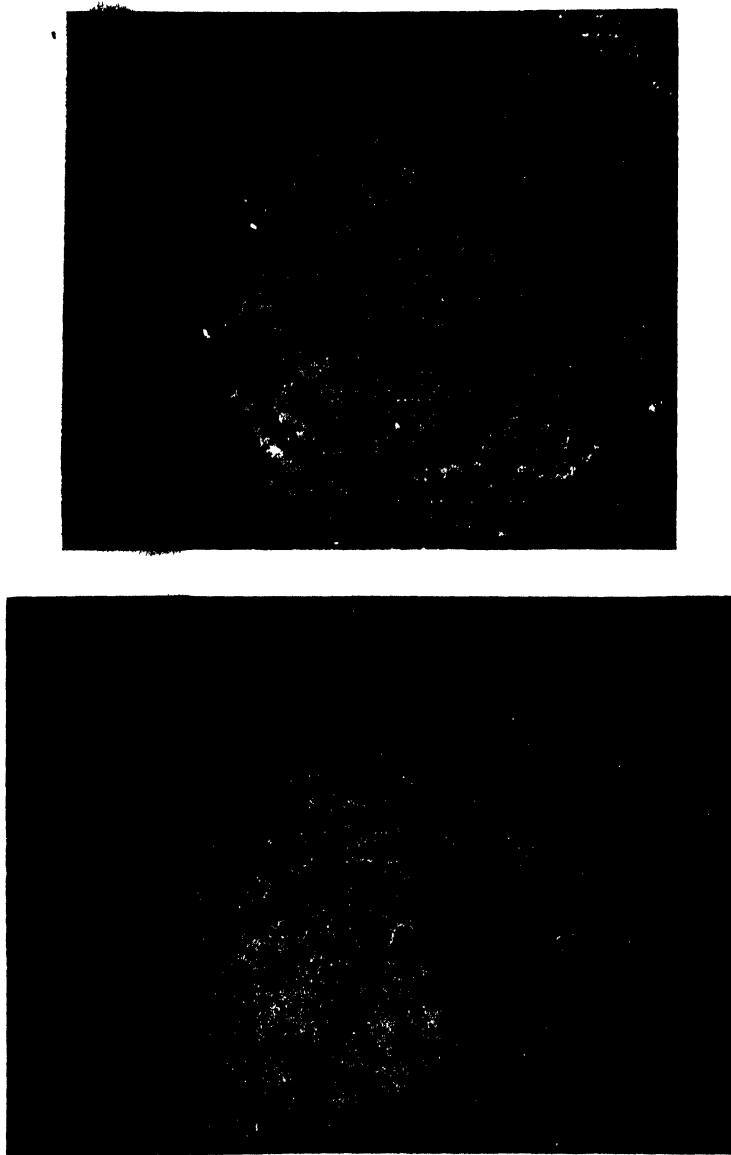


FIG. 4
Synthetic Cholesterol-Calcium Pigment.
Gall Stones showing Rhythmic Banding. ($\times 10$).

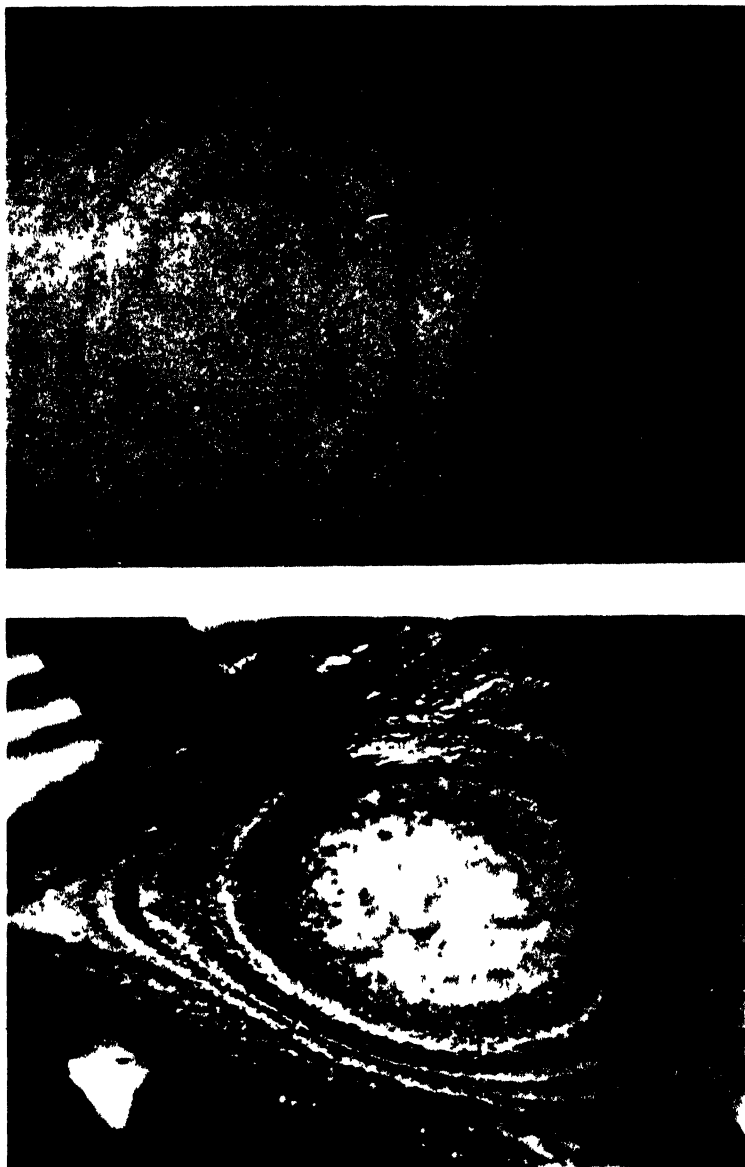


FIG 5

Synthetic Cholesterol Calcium Pigment Stones showing how the Shape of the Stone influences the Form of the Rhythmic Bands ($\times 10$)

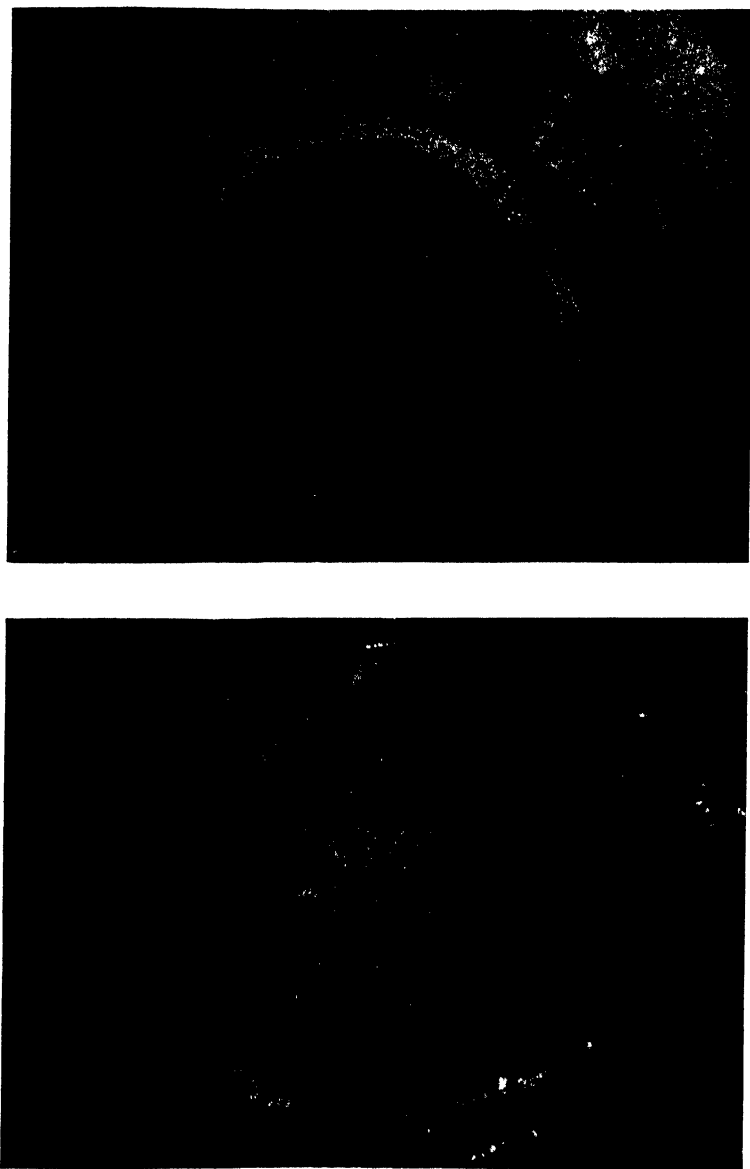


FIG. 6

Synthetic Cholesterol-Calcium Pigment. Gall Stones formed in the presence of a Small Amount of Fibrin.

portions of the latter were placed on a microscope slide and surrounded by the solution of bile pigments. The specimens were placed in a desiccator containing an ammonia solution to prevent their drying out and to minimize the oxidation of bilirubin to biliverdin. After standing over night, concentric rings resulted as shown by the photographs reproduced in Figs. 4 and 5. In Fig. 5 the effect of the shape of the mass on the form of the bands is clearly shown. In order to secure a plane surface to photograph, in some cases a cover glass was pressed down gently on top of the specimen flattening out the high places.

A comparison of the synthetic preparations with the natural gall stones reveals a marked similarity in appearance which indicates that rhythmic banding plays a rôle in the formation of the natural stones just as it does in the synthetic ones.

The objections may be raised that there is no gelatin in natural gall stones. The answer is that the action of gelatin is not specific. It is only necessary that a protective colloid be present to inhibit the growth of the cholesterol crystals and to serve as a kind of binder. Albumin and fibrin, which do occur in natural gall stones may be substituted for gelatin in the formation of banded stones. The specimens shown in Fig. 6 were prepared in the same way as those in Figs. 4 and 5 using instead of gelatin, 50 cc. of water in which was peptized 0.1 to 0.2 gram of fibrin.

Conclusions

From a survey of the conditions which result in the formation of the so-called "layered" stones and the experimental results given in the previous section, it would appear that the formation of such concretions is favored or initiated by inflammation, which yields irreversibly precipitated protein materials, such as fibrin and albumin. Pathologic changes taking about the precipitation of the cholesterol, carrying calcium with it, the nature of the precipitate depending upon the amount of hydrophilic colloid present. Into this mass the colloiddally dispersed bile pigments diffuse and are precipitated in the form of rhythmic bands. The structure and arrangement of the bands is influenced by the shape of the mass, its density due to the presence of other stones, and by variations in the composition of the bile fluid. After the bands are formed the structure may be invaded by radial crystallization of the cholesterol, cracks may develop, further deposition of the cholesterol may occur, or the stone may undergo alteration in other ways, producing the wide variety of forms which are found in gall bladder disease.

Summary

The following is a brief summary of the results of this paper:

1. Rhythmic rings of calcium-bile pigment and of silver chromate were obtained in a mass of cholesterol crystals precipitated in the presence of a small amount of hydrophilic colloid material such as gelatin, albumin, and fibrin.

2. The concentric rings in the "common gall stones" of inflammatory origin are not the result of the deposition of alternate light and dark colored layers but are a manifestation of the Liesegang phenomenon.

3. The colloidal bile pigments diffuse into a mass of cholesterol crystals, hydrophilic colloids and lime and the calcium-bile pigment complex is deposited in concentric bands. The term "layered stone" as applied to the "common gall stone" is a misnomer.

4. Concentric bands are not formed in either natural or synthetic cholesterol stones in the absence of hydrophilic colloids.

5. The structure and arrangement of the bands in both synthetic and natural gall stones is influenced by the density and shape of the mass, the nature and amount of hydrophilic colloid present and the composition of the bile fluid.

6. A survey has been given of the role of colloidal behavior in the formation of the so-called "pure cholesterol" and "pure pigment" stones resulting without inflammation and of the mixed colloid-crystalline stones of inflammatory origin.

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THE MICROTOME METHOD OF THE DETERMINATION OF THE ABSOLUTE AMOUNT OF ADSORPTION

BY JAMES W. McBAIN AND C. W. HUMPHREYS

This work has been concerned with the creation of a microtome method for the determination of the absolute amount of adsorption at *static* air-water interfaces, for which no previous method existed, and with the development of this method to the point where it gives sufficiently accurate and reliable results to serve as a crucial test of the Gibbs adsorption theorem.

The validity of the Gibbs equation has never been demonstrated experimentally. In fact, as pointed out by McBain and DuBois,¹ practically all previous experimental work, where there was not an inherent error in the method used, has given observed values of the adsorption which are considerably in excess of those calculated by means of that thermodynamic equation, either in its strict form or in the approximate form commonly used. The work of McBain, Davies² and DuBois, corroborated by the single measurement of Harkins and Gans,³ shows quite definitely that moving surfaces carry several times more solute than is compatible with the Gibbs equation. However, these moving surfaces, which were used in all previous measurements, may not have represented perfect equilibrium nor have met all of the conditions of the Gibbs equation. Because of the wide use of that equation, its fundamental nature, and the lack of experimental proof of it, measurements of the absolute amount of adsorption at *static* air-water interfaces are plainly needed.

The method which has been developed in this work may be outlined briefly as follows. The solution being studied is kept at rest for any desired length of time in a shallow trough of pure silver surrounded by a saturated atmosphere. By paraffining the ends of the trough the solution is made to bulge up above them without overflowing. A uniform layer 0.05 to 0.1 mm. thick is cut off from a known area (310 sq. cm.) of the surface by a small microtome blade traveling at a speed of about 35 ft. per second. This thin layer of solution is collected in a small silver-lined cylinder on which the microtome blade is mounted. The solution so obtained is weighed and its concentration is compared with that of the bulk of the solution in the trough by means of a Zeiss interferometer. From the observed difference in concentration, the absolute amount of adsorption at the surface of the solution can be calculated.

The Trough, Tracks and Microtome

The main features of the apparatus designed for this work are shown in detail in the accompanying drawings and photographs.

¹ J. W. McBain and R. DuBois: J. Am. Chem. Soc., **51**, 3534 (1929).

² J. W. McBain and G. P. Davies: J. Am. Chem. Soc., **49**, 2230 (1927).

³ W. D. Harkins and D. M. Gans: Colloid Symposium Monograph, **6**, 26 (1929).

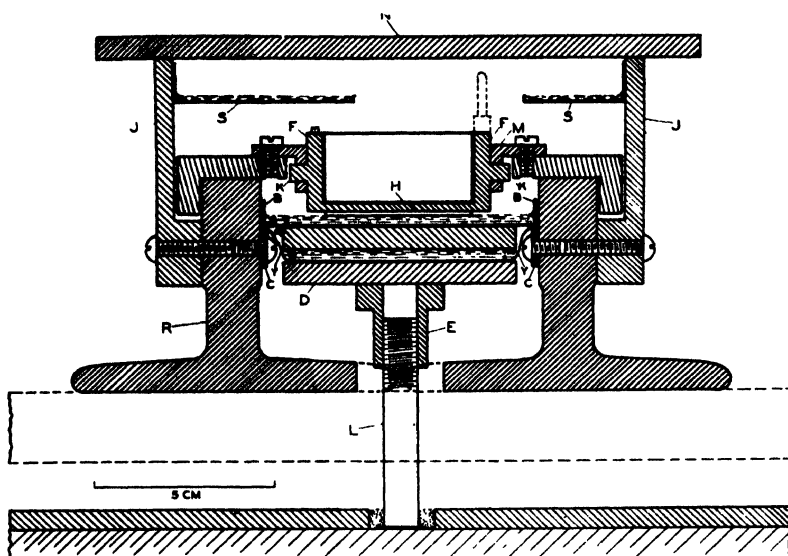


FIG 1 CROSS SECTION THROUGH CENTER OF INNER ENCLOSURE (THROUGH A-A' FIG 3a)

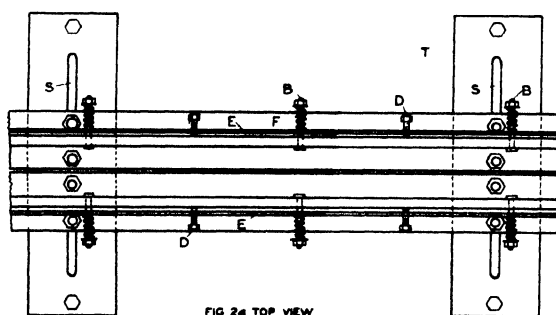


FIG 2a TOP VIEW

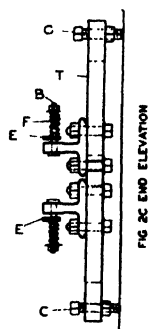


FIG 2c END ELEVATION

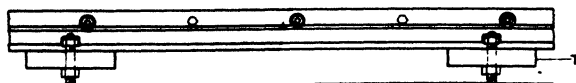


FIG 2b SIDE ELEVATION

THREE VIEWS OF A SECTION OF THE TRACKS SHOWING TRACKS AND BRAKE IN DETAIL

FIG 2

The trough B, Fig. 1, which contains the solution being used is made of pure silver 0.5 millimeters thick. It is approximately 7.5 centimeters wide, 85 centimeters long and the ends and sides are respectively three and eight millimeters high. It is supported between two heavy steel rails, R, as shown in Fig. 1. These rails are thirty-two feet long and it is upon them that the microtome carriage, which supports the microtome blade, slides. The tracks are fastened securely to steel ties at intervals of two feet as shown in Fig. 2. Near the outer edge of each tie is a set screw, C, which rests upon the concrete floor and allows adjusting of the height and leveling of the tracks. The machined upper surface of the rails was sufficiently true for the major part of the tracks. For the part adjoining the trough a much finer adjustment was necessary and was obtained by lapping this portion true so that the variation in level over the whole length of the trough was less than 0.015 mm. The upper part of both sides of the tracks are also machined.

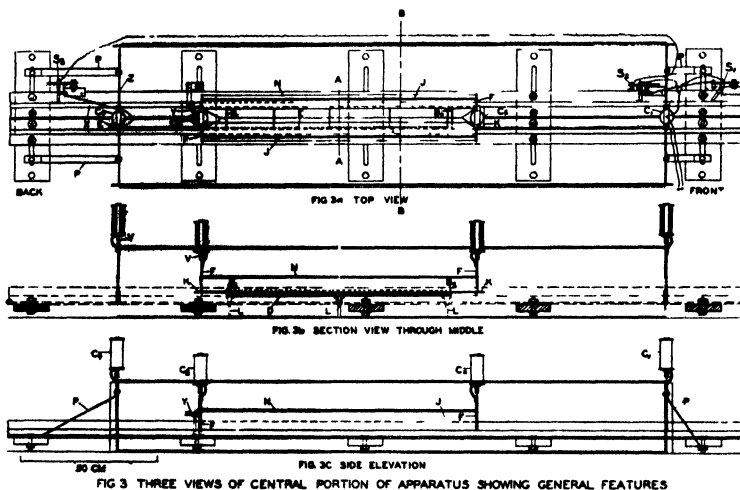
The microtome carriage which slides along the tracks and carries the microtome blade which does the cutting is shown in detail in Fig. 6. It consists essentially of a frame, B, upon which is mounted the cylinder, C, which holds the blade. The cylinder consists of a section of one inch brass tubing, H, which is soldered at the ends to two brass disks. These disks are made with bearings, K, which fit closely through the angle pieces, M, thus allowing the cylinder to turn. The angle pieces are fastened to the carriage so that the cylinder is held firmly in place. A cover, J, made of one-half millimeter sheet silver is fastened over the remaining open part of the cylinder and curves around inside of it, thus acting as a baffle plate to keep the collected liquid from flowing out again. The microtome blade, N, is similar to a rigid safety razor blade. It fits into a small slot milled in the cylinder, as shown in Fig. 6d, and is soldered rigidly in place. The cylinder is lined with pure sheet silver and has a thin coating of paraffin so that the solution collected can be poured out more readily. The solution is removed by pouring it through one side of the same opening through which it enters. The cylinder is held in the proper position for cutting by the small steel pin, E, Fig. 6a, which rests upon the stop, S, and is held down by a thin strip of spring steel, F.

The microtome blade is given the speed necessary for cutting such a thin layer from the surface of the solution by shooting the carriage along the tracks by means of a slingshot arrangement made of rubber tubing. Since it is traveling quite rapidly (at the rate of 25 miles per hour) the carriage must be stopped rather rapidly after passing the trough. To retain the collected liquid the cylinder is turned through about 120° so that the blade and opening are pointing directly upward as soon as the trough is passed. It is turned by the steel pin, P, striking the stationary device, V, Figs. 3a and 3c, and is held in this second position by a small braking device, D and E, Fig. 6c, which has a groove in which the steel pin, P, fits.

The microtome carriage is stopped quickly but without any sudden shock by the brake shown in detail in Fig. 2. This consists of two steel strips, ten feet long, fastened to the outer sides of the rails. The braking action is exerted upon the two sides of the microtome carriage, O, Fig. 6c, which slide in the

groove between the steel strips and the rails. The desired braking action is obtained by adjusting the pressure on the sides by turning the nuts on the ends of the bolts, B, which press upon the springs, F, and by regulating the width of the groove with the set screws D.

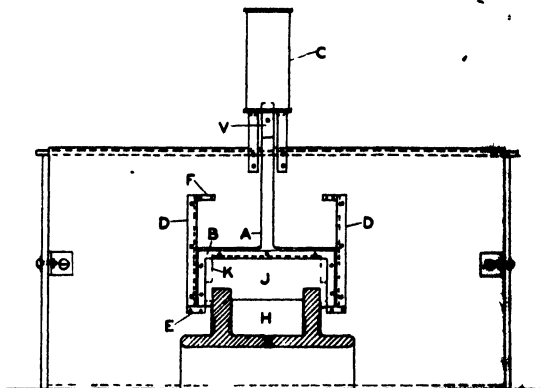
The device, E, Fig. 3a, for stirring the solution in the trough consists of a long strip of silver, one-half millimeter thick and about two millimeters wide, just above the level of the sides of the trough, to which are fastened four cross pieces of the same material which rest upon the bottom of the trough. This long strip extends out beyond the back door and the solution is stirred by merely sliding it back and forth. The stirrer does not break the surface.



The Saturated Vapor surrounding the Trough

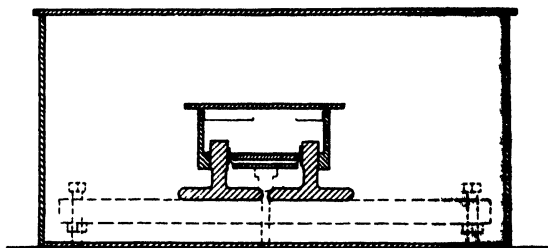
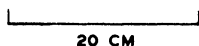
Since any evaporation from the solution in the trough must take place through the surface, its effect would be enormously magnified in the thin layer cut off in an experiment. Therefore it is necessary to prevent any such evaporation from taking place. The apparatus for doing this is shown in cross section in Figs. 1 and 5 and in general layout in Fig. 3. The silver trough is in an inner enclosure which is just large enough in cross section to allow the microtome carriage to pass through and extends about ten centimeters beyond each end of the trough. Around this is a much larger, plate glass, outer enclosure as shown in Figs. 3, 4 and 5, and Photographs 7 and 8. Both enclosures are nearly air-tight. The inner one is sealed off underneath the trough by the sheet of 0.001 inch thick silver foil, V, Fig. 1, which is clamped against the rails by the monel metal strips C and extends down across and upon the plate glass D, underneath the glass strips which separate the glass supports A and D. At the ends, the silver foil comes up and extends out over the ends of the silver-plated brass plates, K, Figs. 3a and 3b, upon which the doors at the ends rest. The sides of the inner enclosure are silver plated brass pieces, J, bolted to the tracks and milled out so that the microtome

carriage can pass through as shown in Fig. 1. The cover, N, is of plate glass. Vaseline is placed between the side pieces and the cover to give an air-tight fit. The sides, top and bottom of the outer enclosure are of plate glass and the ends are of silver plated brass. All exposed brass parts in either enclosure are



END VIEW OF OUTER ENCLOSURE SHOWING DETAILS OF DOOR

FIG 4

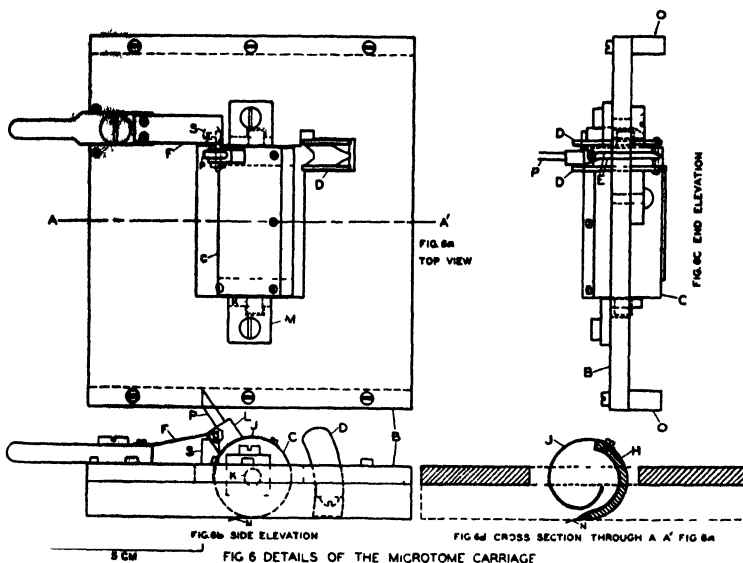


CROSS SECTION THROUGH TRACKS AND BOTH ENCLOSURES
(THROUGH B-B' FIG 3a)

FIG 5

silver plated to limit corrosion; such surfaces come into contact only with vapor. The solution being studied comes into contact only with pure silver or, for brief periods, with glass.

To help saturate the air space in the inner enclosure are two side trays, S, Fig. 1, filled with the solution being used. In addition, the space above the silver foil between the glass supports A and D is filled with the same solution as shown in Fig. 1. A stream of air or nitrogen saturated with respect to the



solution being used, by means of a rocking Washburn¹ saturator, may be passed into the inner enclosure also. In the large outer enclosure are a large number of crystallizing dishes, as shown in Figs. 7 and 8, which contain solution to saturate this outer enclosed air space. There are holes in the covers of both enclosures so that the whole apparatus can be set up and the solution then be put in. These holes are closed with cover glasses.

The Automatic Doors

Since the microtome carriage must pass through both enclosures in making a run, there is a close-fitting door in each end of each enclosure. These doors are automatically opened and closed by electrical contacts which are operated by the carriage itself when it is shot along the tracks. The front door to the outer enclosure is opened first and it is then closed as the other three open so there can be no rush of outside air through the apparatus to cause evaporation. A drawing of one of the doors is shown in Fig. 4 and the photograph in Fig. 8 shows quite well how they are made. A hole is cut through each of the brass

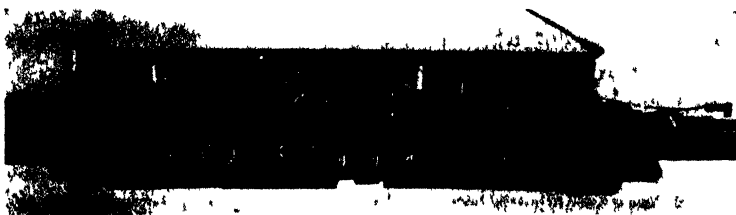


FIG. 7

¹ E. W. Washburn and E. D. Heuse J. Am. Chem. Soc., 37, 309 (1915)

end pieces large enough to let the car through as indicated by the dotted line K in Fig. 4. The frame for the door is very light, being made of a piece of $1/32$ inch duralumin A fastened to a piece of $1/16$ inch duralumin B by small set screws. The frame is large enough so that the microtome carriage can pass through without touching it. It fits as closely as possible against the end piece and still moves freely. Clamped between these two parts of the frame is a piece of silver foil 0.002 inch thick which is cut out so that it fits closely around the tracks and down upon the brass block H, thus closing the opening

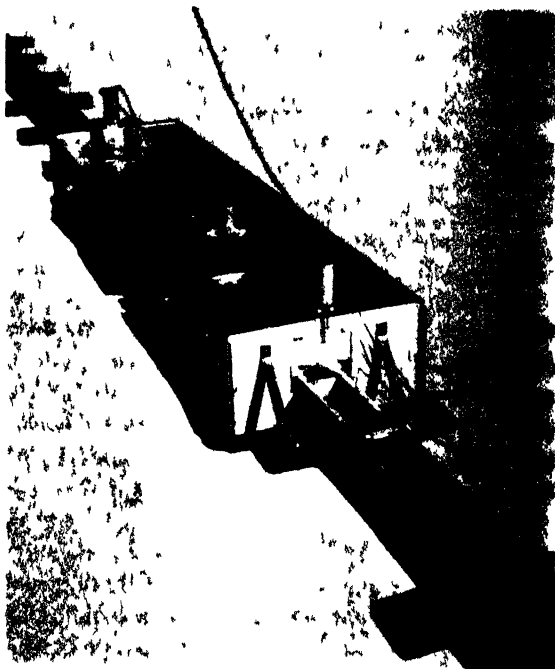


FIG. 8

in the end piece. With the doors to the inner enclosure, the silver foil fits down upon the brass plates, K, Fig. 3, covered with the 0.001 inch silver foil. The doors are made of foil so that, in case they fail to open, the microtome carriage will go right through and thus not wreck any other part of the apparatus or the carriage itself. The door slides up and down in grooves milled out under the brass strips D, Fig. 4. F and E are stops.

To the top of the door frame is fastened a short piece of steel tubing, V, Figs. 4 and 3b. A coil C is fastened above this so that when current is passed through the coil, the door is raised quickly. Each coil consists of about 2400 turns of No. 24 cotton covered copper wire and the four of them are connected in parallel to the 110 volt A.C. line. The microtome carriage operates the switches, S_1 , S_2 and S_3 , Fig. 3, when it is shot along the tracks and thus opens and closes the doors automatically.

Mode of Experiment

A ~~single~~ solution may remain in the trough for weeks or months and be subjected to ~~varied~~ experiments.

A ~~single~~ experiment is conducted as follows. About five to fifteen minutes after the ~~last~~ previous experiment, the amount of solution that was cut off in that ~~run~~, ~~was~~ removed for analysis, is replaced and the solution in the trough is stirred ~~thoroughly~~. It is then allowed to stand, for the gaseous phase to come into equilibrium with the solution, for a known period of time which, in the ~~actual~~ experiments, has varied from about twenty minutes to several days. The ~~thin~~ surface layer is either cut off at the end of this time or else the ~~solution~~ is again stirred thoroughly and allowed to stand for a known short length of time and the sample then cut off. Just before shooting the microtome carriage the cylinder is rinsed twice with solution of very nearly the same concentration as that in the trough. The run is then made as soon as possible after the second rinsing in order to allow as little evaporation as possible from any drops which may remain in the cylinder. The sample cut off is then emptied into a small weighing bottle as soon as possible. The weighing bottle has in it some of the same solution as was used for rinsing the cylinder, and this is emptied out just before the sample is poured in. It is weighed to the nearest tenth of a gram and the sample then poured into the left side of the interferometer cell. The solution in the trough is again stirred and a ten cubic centimeter sample pipetted out with which to rinse and fill the right side of the interferometer cell. The observed difference in concentration, the known weight of the sample and the known surface area cut out give necessary data for calculating the absolute amount of adsorption at the air-solution interface.

With each solution, a series of blank runs was made. A blank run is the same in every detail as a regular run except that the sample, instead of being cut off from the surface of the solution in the trough, is pipetted into the cylinder and shaken around immediately after shooting it along the tracks. This solution is then compared with a sample of the original solution. If there is a change in concentration for any solution a corresponding correction is made in the regular runs.

Calculation of Results

In a single experiment, the following quantities are measured: the weight of solution cut off (W_c); the difference in concentration (I), in grams of solute per gram of water, between the solution cut off and the bulk of the solution in the trough; and the total surface (S) cut out. Knowing the concentration of the solution in the trough (R_o), in grams of solute per gram of water, the adsorption (Γ) can be calculated by the equation:

$$\Gamma = \frac{W_c I}{I + R_o + I} \cdot \frac{1}{S}$$

At concentrations less than ten grams per thousand grams of water this may be reduced to $\Gamma = W_c I/S$ with a resulting error of less than one per cent.

Conditions for a Rigorous Measurement

The ideal conditions for conducting experiments using this method would be those under which the concentration of the solution in the trough would remain constant for an indefinite period of time. It might then be assumed that any difference in concentration between the sample cut off from the surface and the bulk of the solution in the trough was due to adsorption at the air-solution interface, provided there were no additional factors being introduced after cutting off the sample, as shown by blank runs. Such conditions were substantially obtained with one of the solutions studied when the concentration of the solution in the trough remained constant within about two parts per million over a period of twenty-five days during which many observations were made upon it.

It is probably correct to assume that if the concentration of the solution in the trough were increasing with time, any experiment carried out during that period would give a higher value of the adsorption than if the conditions were ideal. Conversely, if the concentration of the solution in the trough were decreasing, any experiment performed during that time would give too low a value of the adsorption. Therefore, if two series of experiments were carried out with one solution so that in one of them the conditions were such that the concentration of the solution in the trough increased very slowly and in the other it decreased very slowly, the value of the adsorption under ideal conditions would probably be between the two values so obtained.

It has been found possible to make the concentration of the solution in the trough either increase or decrease slowly with any of the solutions used by properly regulating the concentration of the solution in the trays, S, Fig. 1, with respect to that of the solution in the trough, and of that in the Washburn saturators when saturated nitrogen is passed into the enclosure. This second method therefore can be used when it is impossible to maintain ideal conditions with a solution of any substance. It was necessary to use this method in some of the experiments which have been carried out and the two values of the adsorption, obtained with the concentration of the solution in the trough increasing and then decreasing slowly, did not differ greatly.

Experimental Results

Before the addition of the large glass outer enclosure, it was impossible to maintain conditions such that the concentration of the solution in the trough would remain constant over a very long period of time. Therefore it was necessary to use the alternative procedure described above, that of controlling the conditions so that the concentration of the solution in the trough first increased and later decreased very slowly, giving two mean values of the adsorption, one of which was probably higher and the other lower than the one which would have been obtained under ideal conditions.

Since the addition of the outer enclosure, only two series of experiments have been conducted, one with hydrocinnamic acid at a concentration of 1.5 grams per 1000 grams of water and the other with the same substance at a

TABLE I

Hydrocinnamic Acid				1.5 g./1000 g. H ₂ O		
Exp. No.	t ₁	t ₂	Wt. of sample	Interface change	Corrected change	Adsorption Γ g./cm ² $\times 10^4$
1	—	22 hrs.	2.7	1.6	1.3	5.2
2	—	3½ hrs.	2.0	2.0	1.6	4.7
3	1 hr.	2 min.	2.2	1.7	1.4	4.6
4	50 min.	2 min.	2.6	1.9	1.6	6.2
5	—	4½ hrs.	2.2	2.1	1.7	5.5
6	50 min.	10 min.	1.7	2.9	2.4	6.0
7	—	11½ hrs.	2.4	1.9	1.5	5.3
8	—	2 hrs.	2.1	1.8	1.5	4.7
9	—	4¼ hrs.	2.4	1.8	1.4	5.0
10	—	12½ hrs.	2.1	2.7	2.3	7.2
11	—	7½ hrs.	2.5	2.0	1.6	5.9
12	—	4¾ hrs.	2.1	1.9	1.5	4.7
13	—	19¼ hrs.	2.4	2.9	2.5	8.9
14	—	18 hrs.	2.4	1.9	1.5	5.3
15	—	24 hrs.	2.4	1.1	0.7	2.5
16	—	47 hrs.	2.2	1.9	1.5	4.9
17	—	4¼ hrs.	2.4	2.0	1.6	5.7
18	—	7 hrs.	2.8	2.0	1.7	7.0
19	—	11¼ hrs.	2.2	2.4	2.0	6.5
20	—	12 hrs.	2.3	2.0	1.6	5.5
21	—	13½ hrs.	2.1	3.0	2.6	8.1
22	—	33½ hrs.	2.2	2.1	1.7	5.5
23	—	21 hrs.	2.4	1.9	1.5	5.3
24	—	65½ hrs.	2.4	2.0	1.7	6.0
25	—	22 hrs.	2.6	2.0	1.7	6.3
2 cm. cell						Ave. = 5.7
26	—	24½ hrs.	2.6	7.2	6.5	12.1
27	—	5 hrs.	2.3	2.3	1.5	2.5
28	—	5 hrs.	2.5	2.8	2.1	3.8
29	—	14 hrs.	2.5	2.7	2.0	3.6
30	—	25 hrs.	2.7	3.6	2.9	5.6
31	—	25 hrs.	3.1	3.0	2.4	5.3
32	—	33¼ hrs.	3.1	2.8	2.2	4.9
33	—	81 hrs.	2.8	3.1	2.5	5.0
4 cm. glass cell						Ave. = 5.4
						Total Ave. = 5.6

concentration of 4.5 grams per 1000 grams of water. In the latter series of experiments, the concentration of the solution in the trough decreased steadily throughout, but only slowly. At the lower concentration, however, the conditions may be considered ideal, since the concentration of the solution in

the trough remained constant within about two parts per million over a period of four weeks. The results of this most satisfactory series of experiments to date are given in detail in Table I.

t_1 refers to the time between when the solution in the trough was last stirred after the preceding experiment and the time it was stirred shortly before that run which is under consideration. t_2 refers to the time elapsing between this second stirring and the time the sample was collected. In most of the experiments in the above table, the solution was not stirred a few minutes before the run was made. In those cases, the time between the last stirring after the preceding run and the time the run being considered was made is listed under t_2 . With each solution, t_1 has had values from one or two minutes to at least several hours. None of them have shown any variation in adsorption depending upon this time interval in which the measured adsorption was allowed to take place.

The "interferometer change" is the difference between the zero reading and the reading obtained by comparing the sample collected with the bulk of the solution in the trough. The "corrected change" is obtained from the "interferometer change" by applying the correction for a blank run. With hydrocinnamic acid at the above concentration, the correction was to subtract 0.3 divisions from the observed interferometer change with the two centimeter cell and 0.6 divisions with the four centimeter cell, and in the case of a 3.0 gram sample, and proportionally more or less for a smaller or larger sample.

The last column gives the adsorption Γ at the air-solution interface as calculated from the observed weight of sample and increase in concentration of the sample over the trough solution, the surface cut out being taken as 310 square centimeters.

With the two centimeter interferometer cell which was used, a change in reading of one division with hydrocinnamic acid solution is equivalent to a change in concentration of 4.50×10^{-6} grams per gram of water. With the four centimeter cell a change of one division is equivalent to a change of 2.22×10^{-6} grams per gram of water.

The results of the above series of experiments are given in such detail to show the nature of the data obtained. They show that, under good conditions, quite consistent results can be obtained with this method.

Since no data on surface tensions of aqueous solutions of hydrocinnamic acid were recorded in the literature, such measurements were carried out by C. Bacon working in this laboratory. He used the drop-weight method as developed by Harkins and his collaborators. From these data, the values of the adsorption predicted by the approximate Gibbs equation at different concentrations were calculated.

Before the large outer enclosure was added, a great number of experiments were carried out with solutions of p-toluidine, phenol and caproic acid. The average values of the observed adsorption for the different substances at each concentration used are listed in the following table for those series of experiments in which the concentration of the solution in the trough was

changing only very slowly and was under control. In the second column is given the number of individual experiments of which the listed observed adsorption is the average value. For comparison, the corresponding values predicted by the Gibbs equation and the values obtained by McBain, Davies and DuBois, using the "bubble method," are also given. What was happening to the concentration of the solution in the trough is also shown.

TABLE II

Substance	No. of Expts.	Conc. g./1000 g. H ₂ O	Ave. Obs. Γ g./cm ² $\times 10^8$	Pre-dicted Gibbs Γ	McBain and Davies	McBain and DuBois	Conc. of Sol'n in trough
p-Toluidine	11	2.00	6.1	5.2	12.6	11.8	Increasing slowly
p-Toluidine	29	1.76	4.6	4.9			Ave. for both increasing and decreasing slowly
Phenol	18	20.48	4.1	4.8		14.8	Increasing slowly
Caproic Acid	30	2.59	6.8	6.3		16.2	Constant
Caproic Acid	14	3.00	5.1	6.5		16.9	Decreasing slowly
Caproic Acid	43	5.25	6.2	6.3		20.5	Decreasing slowly
Hydrocinnamic Acid	33	1.5	5.6	5.1			Constant for four weeks
Hydrocinnamic Acid	19	4.5	5.4	7.9			Decreasing slowly

As previously stated, the above results are those in which the conditions of the experiments approached most nearly ideal conditions, or in which the concentration changes of the solution in the trough have been reversed so that the results which would have been obtained under ideal conditions could be quite closely approximated.

These results represent the only existing measurements of the absolute amount of adsorption at static air-water interfaces. It can be seen that, under the best conditions, the observed values have agreed quite closely with those predicted by the Gibbs equation rather than with the high values observed by McBain, Davies and DuBois and others, all of whom have been able to study moving surfaces only. They do not represent as large a body of data as that obtained by various workers with moving surfaces. However, under the best conditions, the static surfaces used here certainly approach more nearly the conditions of the Gibbs equation than do dynamic surfaces. The Gibbs equation would thus appear as a limiting law.

This method opens up a wide field for investigation. The preliminary results here reported show the desirability of further measurements of the absolute amount of adsorption at static air-solution interfaces, particularly over a greater range of concentration and with a great variety of solutions of all types.

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May, 1931.

AN ELECTRICAL CONDUCTIVITY METHOD FOR DETERMINING THE EFFECTIVE CAPILLARY DIMENSIONS OF WOOD

BY ALFRED J. STAMM¹

Four dynamic physical methods for studying capillary structure have been developed and applied at the Forest Products Laboratory to the determination of the effective dimensions of the fine, continuous, capillary structure of softwoods.^{2,3} An electroendosmotic flow method was used for determining the effective continuous capillary cross-section of wood. Data obtained from these measurements were combined with data from hydrostatic flow studies for calculation of the average diameters of the effective openings. Although the electroendosmotic method proved to be of considerable use in determining the average and the limiting values of the various fiber lengths, it is too indirect and inaccurate a method for quantitative capillary cross-section determinations. With wood sections of low permeability, in which the resistance to flow was high, measurements of the velocity of electroendosmosis could not be made with greater accuracy than 20 per cent.⁴ The determination of the increase in electroendosmotic velocity with an increase in capillary cross-section introduced another possible error resulting from the assumption that the increase in capillary cross-section caused by the drilling of fine holes in the sections could be calculated from the cross-section of the bit. The calculated increase in cross-section may have differed from the actual increase by as much as 50 per cent because of the distortion of the holes and the unavoidable tearing and brooming of the fibers. Another approximation, that of estimating the length of the effective capillaries, was necessary in combining the electroendosmosis data with data obtained from hydrostatic flow studies in order to obtain values for the average effective capillary diameters. This estimate could be made with an accuracy of approximately 50 per cent. The final calculated diameters were therefore determinable with no greater certainty than a possible error of 100 per cent. This low order of accuracy seems entirely reasonable, however, when it is realized that these openings are in general below microscopic visibility in size. Nevertheless more accurate results were desired for use in connection with a theoretical study of the rate of drying of wood. Because of this need the method described in this paper was developed.

¹ Forest Products Laboratory, Forest Service, U. S. Department of Agriculture maintained at Madison, Wis., in cooperation with the University of Wisconsin.

² Stamm: Colloid Symposium Monograph, 6, 83 (1928).

³ Stamm: J. Agr. Research, 38, 23 (1929).

⁴ O. T. Qumby, working in this Laboratory, found that the inconstancies were due to thermal and polarization effects. The use of non-polarizing electrodes might have given more accurate results, but because of the development of the new method this was never tried.

Structure of Softwoods

Electrical conductivity measurements have been used in the past for determining the ratio of the effective capillary length to the effective capillary cross-section of porous materials.⁵ None of the materials investigated, however, were made up of capillaries of two such distinctly different orders of magnitude as those in wood. Because of this complication a brief consideration of the structure of softwoods is necessary.

The fiber cavities that make up the major part of the void volume of softwoods are closed at both ends, the only communication from fiber cavity to fiber cavity being through the pores in the membranes of the bordered pits. It is the size and the number of these finer openings that control the permeability of wood. Each softwood fiber with an average length of about 0.3 cm. and a diameter of approximately one hundredth of this value has from 30 to 300 pits connecting it with adjoining fibers, and there are from 50,000 to 100,000 such fibers in a square centimeter of cross-section.⁶

Fig. 1 gives a diagrammatic representation of the capillary path through wood, indicating the manner in which the fiber cavities (shown in black) are connected through the pores of the pit membranes.

Method for determining the Ratio of the Effective Capillary Length to the Effective Continuous Capillary Cross-Section

The ratio of the effective capillary length to the effective continuous capillary cross-section can be calculated from the electrical resistance of sections of wood, the voids of which are completely filled with a salt-solution, and from the specific resistance of the salt solution in bulk, providing the surface conductivity is made negligible and the conductance of the cell wall is negligible. The surface conductivity for very dilute salt solutions and especially for distilled water may be many times the bulk conductivity of the solution or of the water,^{2,3,5,7,8} but for salt solutions of appreciable concentration this surface conductivity becomes negligible in comparison with the bulk conductivity. When the potassium chloride solutions used in this investigation exceeded a concentration of 0.07 mol per liter, the ratio of the specific resistance of the salt solution in bulk to its resistance in the wood structure was found to be independent of concentration, thus indicating that the surface effects are negligible. Hence measurements were made using salt solutions exceeding this concentration.

The specific conductance of dry wood is extremely small, approximately 10^{-12} mho for a cube of wood 1 cm. on an edge. When water is adsorbed by the cell wall the conductivity increases so as to give a linear relationship

⁵ Fairbrother and Mastin: *J. Chem. Soc.*, **125**, 2319 (1924); Hitchcock: *J. Gen. Physiol.*, **9**, 755 (1926); Marshall: *J. Soc. Chem. Ind.*, **46**, 373T (1929).

⁶ For a further description of the capillary structure of wood, see Stamm: *Colloid Symposia Monograph*, **4**, 246 (1926).

⁷ D. R. Briggs: *J. Phys. Chem.*, **32**, 641 (1928).

⁸ McBain, Peaker and King: *J. Am. Chem. Soc.*, **51**, 3294 (1929); McBain and Peaker: *J. Phys. Chem.*, **34**, 1033 (1930).

between the logarithm of the conductivity and the moisture content, up to fiber saturation, at which point the specific conductance is 3×10^{-6} to 1×10^{-5} mho.⁹ The water adsorbed in the cell wall at this point shows an increased conductivity over the bulk conductivity of water, because of surface conduction. Salt solutions, however, act differently. The conductivity of such solutions at the fiber-saturation point, although exceeding that of water, is sufficiently less than the bulk conductivity to make the conductivity of the cell wall in the presence of the salt negligible for the present measurements.



FIG. 1
Simplified diagrammatic representation of the capillary path through wood showing the manner in which the fiber cavities are connected through the pores of the pit membranes.

This fact may be illustrated by measurements made upon a transverse section of Douglas fir sapwood. The section when completely filled with a potassium chloride solution (0.199 mol per liter and 39.1 ohms specific resistance) had a resistance of 26.0 ohms. The section contained 1.907 gm. of salt solution and 1.869 gm. of water. When the section was dried to approximately the fiber-saturation point, 29.3 per cent water, the resistance was 5,100 ohms. Although the capillary cross section effective for electrical conduction was reduced in proportion to the liquid lost, the concentration of the salt was increased in the same proportion. The electrical resistance should have remained approximately constant if the liquid in the cell wall were fully as conductive as the free liquid in the cell cavities. The increase in resistance, however, is nearly two hundred fold, showing that the salt solution can not be dispersed in the cell wall in a continuous manner. Neglecting the resistance of the cell wall in parallel with the fiber-cavity resistance introduces an error of not more than 0.5 per cent.

Further evidence that solutes do not become dispersed in the cell wall in a continuous manner as water does, but on the contrary are confined to the grosser capillary spaces, is given in a previous investigation on the effect of solutes upon the apparent density of the wood substance.¹⁰

The electrical conductivity of a transverse wood section, the capillary structure of which is filled with a salt solution, is thus substantially equal to the sum of the bulk conductivities of the solution in all of the individual capillary paths connected in parallel. These capillary paths in turn are made up of fiber cavities and pores of pit membranes connected in series (Fig. 1). The part of the electrical resistance for which the fiber cavities are responsible can be calculated from the fractional void volume, V , which in turn can be calculated from the bulk

⁹ Stamm: Ind. Eng. Chem. Anal. Ed., 1, 94 (1929).

¹⁰ Stamm: J. Phys. Chem., 33, 409 (1929).

density, d , of the wood on a wet volume and dry weight basis, and the density of wood substance, d_o , which is equal to 1.52 gm. per cu. cm. Thus,

$$V = 1 - d/d_o \quad (1)$$

The fractional void volume obtained in this way includes all void structure. For softwoods free from resin ducts this void volume is made up mostly of fiber cavities, together with the water-filled void structure of the swollen cell wall and the void volume of the ray cells. The ray cell voids, which amount to only 1 or 2 per cent of the total, have been neglected to simplify the calculations.

The fractional void volume of the cell wall is equal to the product of the moisture content, M , per gram of dry wood at the fiber-saturation point, and the density of the wood, d . Then the fractional void volume of the fiber cavities per cubic centimeter of wood

$$V_f = V - Md \quad (2)$$

This void volume is made up of a longitudinal component of 1 centimeter, and radial and tangential components that approach equality. The void cross-section of the average fiber cavity for transverse sections

$$q_f = V - Md \quad (3)$$

Then the electrical resistance of the combined fiber cavities

$$R_f = \frac{R_{sp}L}{(V - Md)Q} \quad (4)$$

where R_{sp} is the specific resistance of the salt solution, L the number of unit thicknesses of the section under investigation, and Q the number of unit cross-sections of the specimen.

The total resistance, R_m , of the salt solution in the pores of the pit membranes will depend upon the fractional cross-section of such pores traversed in parallel and the length of path, that is, the continuous effective capillary cross-section, q_m , of the pores expressed as a fraction of the cross-section of the specimen, and the sum, l_m , of the thicknesses of the pit membranes traversed in series. Then,

$$R_m = \frac{R_{sp}l_m}{Q q_m} \quad (5)$$

The experimentally measured resistance, R , is equal to the sum of the resistances from equations (4) and (5).

$$R = \frac{R_{sp}L}{(V - Md)Q} + \frac{R_{sp}l_m}{Q q_m} \quad (6)$$

and

$$\frac{l_m}{q_m} = \frac{Q}{R_{sp}} \left(R - \frac{R_{sp}L}{(V - Md)Q} \right) \quad (7)$$

for transverse sections.

For radial sections, where the flow of current is in the tangential direction, equation (7) takes a somewhat simpler form since

$$R_f = \frac{R_{sp}L}{Q} \frac{\sqrt{V - Md}}{\sqrt{V - Md}} = \frac{R_{sp}L}{Q} \quad (8)$$

and therefore

$$\frac{l_m}{q_m} = \frac{Q}{R_{sp}} \left(R - \frac{R_{sp}L}{Q} \right) \quad (9)$$

Although the radial and the tangential components of the void volume are not exactly equal, they may be considered so for this calculation, since R_f is so small in comparison with R_m that a deviation of 50 per cent in R_f will cause an error in R_m of only 2 per cent.

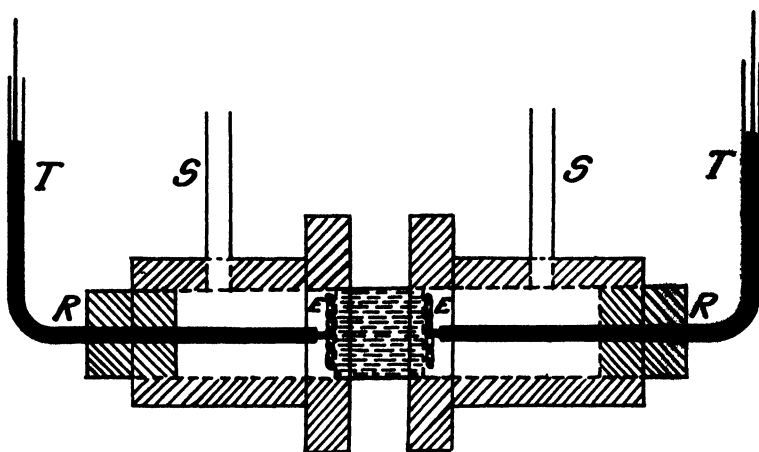


FIG. 2

Cells used in electrical resistance measurements.

The measurements were made upon wood sections that were clamped between the faces of two hard rubber cells and upon cylindrical sections that had been turned in a lathe in a soaked and swollen condition to fit tightly into the ends of the cells (Fig. 2). Several sets of cells and electrodes of different cross-section were used for the measurements. The electrodes, E , were made of heavy platinum wire wound in the form of disk coils and sealed into glass tubes, T , that were filled with mercury for electrical contact. The electrodes were fitted into the cells with rubber stoppers, R . The side tubes, S , served in filling the cells with the salt solutions. The electrodes were freshly platinized each day before using. The measuring apparatus consisted of a student circular slide-wire bridge, a four-dial resistance box (0.1 to 999.9 ohms), a microphone hummer, telephone receivers, a condenser, a switch, and dry cells connected according to standard conductivity measuring practice.

The sections were soaked in distilled water for at least two weeks. To facilitate the replacement of air by water the soaking was done in a vacuum desiccator to which suction was applied intermittently. Potassium chloride

was then added. Electrical resistance measurements on the wood sections showed that diffusion was complete and equilibrium of the salt distribution was obtained in less than a week. The specific resistance of the salt solution in equilibrium with the wood sections was determined, using the hard-rubber cells of Fig. 2 clamped together with a ring gasket replacing the wood section. Measurements were made with the electrodes separated by different distances. All measurements were made at 25° C in a thermostatic air bath.

Experimental Results

The differences between measurements made with broad transverse sections clamped between the faces of the cells and cylindrical sections cut to fit the cells were first investigated. Current in the broad sections fanned out appreciably beyond the area bounded by the cells. The extent of the spreading of the current proved to be a function of the specific electrical conductivity and of the thickness of the sections and was independent of the cross section of the cell. It is thus possible to correct for this spreading of the current for transverse sections when measurements are made on the same specimen with cells of two different cross sections. Although the extent of the spreading will increase from zero at each of the surfaces of the section to a maximum at the center, it is simpler for these calculations to consider an average effective extent of spreading X . This must be added to each of the cell radii in order to calculate the effective cross section. Then,

$$\left(\frac{r_1 + X}{r_2 + X} \right)^2 = \frac{R_2}{R_1} \quad (10)$$

where r_1 and r_2 are the cell radii of two different cells and R_1 and R_2 are the corresponding resistances. Table I gives the specific resistances calculated in this manner, using three different sizes of cells, as well as the results obtained from measurements made on transverse cylindrical sections cut to fit the cells. The values obtained by the two methods agree quite well. There is a slight tendency, however, for the specific resistance to be slightly less for the cut sections; this will be considered again later in this article.

Measurements made in equilibrium with two different concentrations of salt solution are given in Table II. The results agree within experimental error for the concentrations used.

The values of l_m/q_m for different thicknesses of the transverse sections are plotted in Fig. 3 for all of the Sitka spruce data given in Table I and Table II, together with data for two other Sitka spruce specimens of different density and two species of cedar. The data in all cases show a linear relationship between l_m/q_m and the thickness of the section; the graphs when extrapolated to zero thickness pass through the origin. This indicates that the continuous effective capillary cross section, q_m , varies but slightly for adjoining sections and that the length of the effective capillaries, l_m , varies directly with the thickness of the section, thus indicating a rather uniform distribution of

TABLE I

Comparison of the Specific Electrical Resistance of Transverse Sections of previously Seasoned and Resoaked Sitka Spruce calculated from Measurements made upon Cylindrical Sections cut to fit the Cells and Sections extending beyond the Cell Cross-Sections.

Density of the wood (volume green and weight oven-dry)—0.297 gm. per cu. cm.

Concentration of potassium chloride solution—0.81 mol per liter

Specific resistance of potassium chloride solution—10.31 ohms

Kind of section	Thick-ness of section	Radius of cell	Meas-ured resistance	Extent of spread-ing X	Effective area of cross section	Calculated specific resistance of section	l_m/q_m
	<i>Cm.</i>	<i>Cm.</i>	<i>Ohms.</i>	<i>Cm.</i>	<i>Cm.²</i>	<i>Ohms.</i>	
Extending	0.615	0.870	4.01	0.0398	2.600	16.96	0.151
		.430	15.00	.0398	.694	16.92	
		.245	40.80	.0399	.255	16.92	
	1.062	.870	6.00	.1030	2.974	16.80	.248
		.430	20.00	.1037	.896	16.87	
		.245	46.80	.1037	.382	16.82	
	1.481	.870	7.75	.137	3.186	16.70	.333
		.430	24.50	.137	1.010	16.70	
Cut	1.481	.870	10.40	.000	2.378	16.68	.323
Extending	2.031	.870	10.48	.156	3.307	17.07	.523
		.430	32.10	.156	1.079	17.05	
Cut	2.031	.870	14.40	.000	2.378	16.85	.484
		.430	58.80	.000	.581	16.82	.480

equally unobstructed capillaries. Such a relationship is rather to be expected, considering the large number of fiber cavities and pit membranes traversed by the current.

The ratio l_m/q_m per unit thickness for the specimens of Sitka spruce of different density varies directly with the density (Fig. 3 and Table III). The simplest explanation of this relationship is that the pit membrane thicknesses vary directly with the density, while q_m remains practically constant. Values of q_m , however, will vary from species to species.

Fig. 4 and Table IV show the differences in the ratio l_m/q_m for sapwood and heartwood. The differences in effective capillary cross section are surprisingly small, thus indicating that the large differences in the permeability of sapwood and heartwood are due to another cause. This matter will be considered in more detail later in this article.

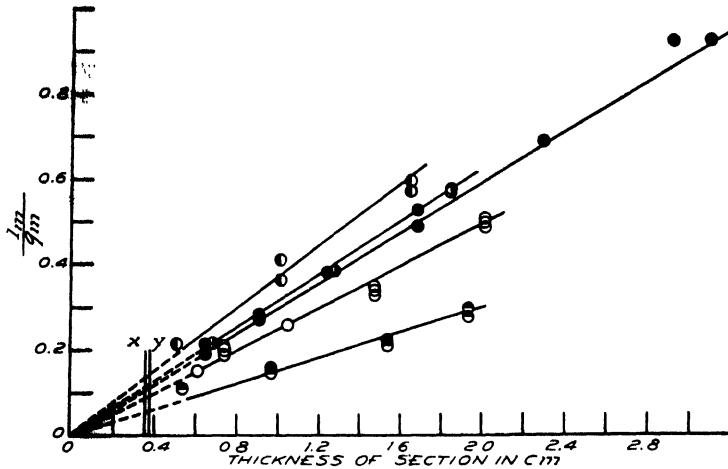


FIG. 3

Relationship between the ratio of the effective capillary length to the effective continuous capillary cross section, and the thickness of the section, for spruce and cedar.

- Sitka spruce, density 0.297 gm. per cu. cm.
- Sitka spruce, density 0.347 gm. per cu. cm.
- ◐ Sitka spruce, density 0.370 gm. per cu. cm.
- ◑ Alaska cedar, density 0.442 gm. per cu. cm.
- ◒ Western red cedar, density 0.290 gm. per cu. cm.
- x Average fiber length for Alaska cedar
- y Average fiber length for Sitka spruce and western red cedar

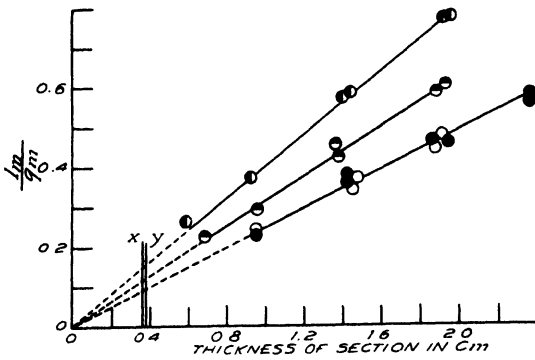


FIG. 4

Relationship between the ratio of the effective capillary length to the effective continuous capillary cross section, and the thickness of the section, for Douglas fir and slash pine.

- Coast Douglas fir, sapwood
- Coast Douglas fir, heartwood
- ◐ Slash pine, sapwood
- ◑ Slash pine, heartwood
- x Average fiber length for pine
- y Average fiber length for Douglas fir

TABLE II

Measurements made in Equilibrium with Two Different Concentrations of Salt Solution upon previously Seasoned and Resoaked Transverse Sections of Sitka Spruce cut to fit the Cells.

Density of the wood (volume green and weight oven-dry)—0.297 gm. per cu. cm.

Thickness of section	Radius of section	Concentration of potassium chloride in mols per liter	Specific resistance of potassium chloride solution	Resistance of section	l_m/q_m
<i>Cm.</i>	<i>Cm.</i>		<i>Ohms.</i>	<i>Ohms.</i>	
0.751	0.870	0.810	10.31	5.4	0.196
	.430	.810	10.31	22.1	.194
	.870	.134	58.00	30.4	.197
	.430	.134	58.00	123.7	.187
1.481	.870	.810	10.31	10.4	.323
	.870	.134	58.00	58.8	.340
	.430	.134	58.00	240.5	.335
2.031	.870	.810	10.31	14.4	.484
	.430	.810	10.31	58.8	.480
	.870	.134	58.00	81.3	.492
	.430	.134	58.00	332.0	.491

TABLE III

Effect of the Density of previously Seasoned and Resoaked Sitka Spruce upon the Ratio of the Effective Capillary Length to the Effective Continuous Capillary Cross Section

Density of specimen	l_m/q_m for average fiber length	Column 2 values Column 1 values
$\frac{\text{Gm.}}{\text{Cm.}^3}$		
0.297	0.091	0.306
.347	.108	.312
.370	.115	.311

TABLE IV

Comparison of Measurements Made upon Unseasoned Transverse and Radial Sections of the Sapwood and the Heartwood of Slash Pine

Sapwood, density (volume green and weight oven-dry)—0.456 gm. per cu. cm.
Average number of fibers and rays traversed per mm. in the tangential direction—34.0

Heartwood, density (volume green and weight oven-dry)—0.430 gm. per cu. cm.
Average number of fibers and rays traversed per mm. in the tangential direction—33.0

Concentration of potassium chloride solution—0.79 mol per liter

Specific resistance of potassium chloride solution—9.84 ohms

Part of wood	Section	Thickness of section	Radius of section	Resistance of section	l_m/q_m	l_m/q_m per pit membrane traversed in series	
		<i>Cm.</i>	<i>Cm.</i>			Observed	Corrected
Sap	Transverse	1.940	0.92	15.00	0.608		
		1.365	.92	10.65	.453		
		.960	.92	7.38	.292		
		.640	.92	5.05	.229		¹ 0.055
		1.890	.46	58.50	.588		
		1.395	.46	43.00	.426		
Heart	Transverse	1.965	.92	15.30	.783		
		1.450	.92	11.30	.587		
		.922	.92	7.20	.375		
		.585	.92	4.68	.267		¹ 0.070
		1.925	.46	59.90	.771		
		1.400	.46	43.80	.575		
Sap	Radial	1.830	0.92	163.0	42.2	0.0677	
		.750	.92	68.7	17.8	.0698	² 0.0810
		1.380	.46	416.0	26.8	.0571	
		.970	.46	306.0	19.7	.0578	
Heart	Radial	1.680	.92	158.5	41.2	.0743	
		1.240	.92	118.5	30.8	.0752	² 0.0927
		1.230	.46	391.0	25.2	.0622	
		.900	.46	289.0	18.6	.0627	

¹ For a travel of half the average fiber length; taken from Fig. 3.

² For the average diameter of the fibers and rays traversed, corrected for leakage of surface current across the sections.

Table IV gives also the data for radial sections of both the sapwood and the heartwood of slash pine. The values for l_m/q_m in this case were obtained from equation (9). The number of fibers and ray cells traversed in the tangential direction per unit of distance was determined by direct microscopic

measurements upon transverse microtome sections.¹¹ The values of l_m/q_m per membrane traversed in series for the sections turned on a lathe so as to fit the cells vary with the radius of the cell, because of a slight leakage of current over the cylindrical surface of the sections. Assuming the thickness of this leakage film to be the same for both cells, the total leakage current will vary directly as the circumference and consequently as the radius of the cell. The leakage per unit of cross section, however, will vary inversely as the radius of the cell. Therefore,

$$\frac{1}{R_1} - C = \frac{1}{R_2} - \frac{r_1}{r_2} C \quad (11)$$

in which R_1 and R_2 are the measured resistances reduced to unit dimensions for the cells with radii r_1 and r_2 , respectively, and C is the leakage conductance for r_1 . This conductance, in general, amounts to 18 to 20 per cent of the total conductance. Presumably there is a similar surface leakage of current with the transverse sections previously considered, but because the resistance of those sections is less than a tenth of the resistance of the radial sections, the error will be less than 2 per cent. This fact will account for the tendency for the measurements on the cut sections given in Table I to be slightly less than those for the broad sections. No measurements were made on broad radial sections because the necessary correction would be complicated by the longitudinal spreading of the current, which is greater than the spreading in the radial direction on account of the difference in conductivity in these two directions.¹²

The reason for the deviations between the values for l_m/q_m per membrane traversed in series for the transverse and the radial sections is that the values for the transverse sections were calculated by assuming a uniform distribution of the pit membranes along the length of the fiber. Only with such distribution does the current pass on the average through no more than one pit membrane in traversing a distance of half a fiber length. Microscopical observations, however, have shown that the pits are much more numerous near the ends of the fibers, thus actually requiring a greater section thickness than half a fiber length for the current to pass on the average through even one pit membrane. The flow conditions can perhaps be better understood by considering the current that passes through the fiber cavities as being made up of a bundle of threads in parallel, each thread entering a fiber cavity through a single pit membrane opening and leaving through an opening in another pit membrane. A thread may enter at one pit and leave through the nearest pit or it may enter through a pit close to one end of the fiber and leave through a pit at the other end. Hence the possible paths for these filament currents vary from a negligibly short length to practically the full fiber length; the actual location of the pits, of course, gives an average length of path greater than the uniform location assumed.

¹¹ These measurements are further described in "A New Method for determining the Proportion of the Length of a Tracheid that is in Contact with Rays," by Stamm, *Botanical Gazette*, 92, 101 (1931).

¹² Stamm: *Ind. Eng. Chem*, 19, 1021 (1927).

An approximation of the actual value of q_m can be made from the value of l_m/q_m for transverse sections taken from the graphs for a section whose thickness is equal to that of half the average fiber length, and from the thickness of a single pit membrane, which was found microscopically to range from 2×10^{-5} to 2×10^{-4} cm.; it would perhaps be better to use l_m/q_m for radial sections having a thickness of the average fiber and ray diameter. Dividing l_m by l_m/q_m gives a value of q_m ranging from 0.3×10^{-3} to 8.0×10^{-3} for all of the species studied. The electroendosmosis method gave values of q_m ranging from 0.9×10^{-3} to 1.7×10^{-3} .^{2,3} This agreement is quite satisfactory when the large uncertainty of the effective value of l_m is considered.

Effective Capillary Dimensions

The preceding data for l_m/q_m were combined with data from hydrostatic flow studies^{2,3,13} to calculate the average effective capillary radii. Measurements were also made by the method for overcoming the effect of the surface tension of water in the capillary system to obtain the maximum effective capillary radii. All of the data are assembled in Table V. The maximum radii range from about 1 to 6 times the average radii. The radii obtained by overcoming the effect of surface tension decrease with an increase in the thickness of the section. This is to be expected; the most effective path through a number of pit membranes in series will approach the average effective path as the number of pit membranes in series increases, because of the decreasing probability of all the pit membranes in series containing pores of maximum size. For example, measurements made upon radial sections, in which the tangential displacement of water by gas is through the same structure, except that far more pit membranes are traversed in series per unit thickness of the section, gave a value of r for the heartwood of slash pine of 4.0×10^{-6} and for the sapwood of 3.7×10^{-4} . The number of pit membranes traversed in series for these sections was approximately 40. The data thus show that the radii obtained by the method of overcoming the effect of surface tension approach the values obtained by the electrical conductivity and the hydrostatic flow method. The fact that these two entirely different methods of measuring the size of the effective openings give results of the same order of magnitude provides confirmation of the validity of the methods.

The data further show the large differences in the effective capillary dimensions for the sapwood and the heartwood of slash pine. The difference between the capillary dimensions of the sapwood and the heartwood of the Douglas fir specimens is much less. This can be partially attributed to the presence of ring shakes, that is, cracks between the annual rings in the heartwood, which tends to increase the heartwood values. Treatment of Douglas fir with creosote indicates that for this species there is an appreciable penetration through ring shakes.

¹³ Stamm: Physics, 1, 116 (1931).

TABLE V
Effective capillary sizes from hydrostatic flow and from overcoming of surface tension

Species of wood	Density of section	Thickness of section	Effective cross section	Standard capillary constant	Extrapolated pressure drop ratio	l_m/q_m (from Fig. 2 and Fig. 3)	Average effective capillary radius	Pressure to overcome surface tension	Maximum effective capillary radius
	ρ_m/Cm^3	L	Q	r_2^2/l_2	P_2/P_1		$Cm.$	$Kg./Cm.^2$	$Cm.$
Slash pine heartwood unseasoned	.430 .430 .430	1.340 .875 .670	2.08 2.08 2.08	0.53×10^{-9} .53 .53 3.73	0.013 .025 .037 .0047	0.540 .350 .270 .270	2.37×10^{-6} 2.65 2.83 2.66	27.0 22.0 15.5 —	5.5×10^{-6} 6.7 9.5 —
Slash pine sapwood unseasoned	.456 .456 .456	1.980 1.365 .915 .730	2.08 2.08 2.08 2.08	32.1 32.1 32.1 32.1	1.25 1.70 2.40 2.85	.620 .430 .290 .230	2.19×10^{-4} 2.11 2.04 1.97	12.0 11.2 10.2 9.5	9.0×10^{-4} 9.7 10.6 11.4
Coast Douglas fir heartwood unseasoned	.326 .326 .326 .326	2.490 1.820 1.480 1.000 .740	1.54 1.54 1.54 1.54 1.54	3.73 3.73 3.73 3.73 3.73	.010 .012 .017 .023 .033	.610 .450 .360 .245 .180	6.83×10^{-6} 6.43 6.83 6.56 6.73	20.5 19.5 19.0 18.0 17.0	7.18×10^{-6} 7.55 7.75 8.18 8.65
Coast Douglas fir sapwood unseasoned	.403 .403 .403	1.830 1.460 .930 .707	1.54 1.54 1.54 1.54	3.73 3.73 3.73 3.73	.10 .13 .20 .27	.450 .360 .235 .175	1.86×10^{-5} 1.89 1.90 1.90	9.0 8.0 7.0 3.5	1.84×10^{-5} 1.84 2.10 4.20
Alaska cedar seasoned and resawed	.442 .442 .442	1.218 .873 .582	2.08 2.08 2.08	1.93 1.93 1.93	.0017 .0035 .0090	.435 .310 .210	1.47×10^{-6} 1.78 2.35	40.0 43.0 37.0	3.7×10^{-6} 3.4 4.0
Western red cedar heartwood seasoned and resawed	.290 .290 .290	1.090 1.067 .795 .610	2.08 2.08 2.08 2.08	.81 1.99 1.99 .81	.010 .0022 .0045 .024	.160 .160 .115 .090	1.40×10^{-6} 1.03 1.25 1.63	— 28.0 21.0 —	— 5.2×10^{-6} 7.0 —
Sitka spruce heartwood seasoned and resawed	.347 .347 .347	1.885 1.130 1.130	2.08 2.08 2.08	3.73 3.73 .53	.0025 .0035 025	.555 .330 .330	2.80×10^{-6} 2.56 2.58	17.0 13.0 —	8.6×10^{-6} 11.3 —

Summary

1. A method has been developed for determining the ratio of the effective capillary length to the effective continuous capillary cross section by means of electrical resistance measurements of salt solutions filling the wood structure, and the resistance of the solutions in bulk.

2. Measurements made upon sections cut to fit the electrode cells and sections extending beyond the effective area of the cells agree when a correction is made for the spreading of the current in the oversize sections.

3. The concentration of the salt solution does not affect the results when it exceeds 0.07 mol per liter.

4. The ratio l_m/q_m for a single species varies directly with the density of the wood.

5. The values of the ratio l_m/q_m do not differ greatly between the sapwood and the heartwood.

6. The values of the ratio l_m/q_m per pit membrane traversed, calculated from measurements made upon tangential and radial sections, agree quite well.

7. Combining the data for l_m/q_m with data obtained from hydrostatic flow studies gives the average radii of the effective capillaries. These values are compared with the maximum effective radii obtained by the method of overcoming the effect of surface tension. The maximum values range from about 1 to 6 times the average values and approach more nearly the average values when the measurements are made under conditions in which a large number of pit membranes are traversed in series. The effective radii of pit membrane pores are larger for sapwood than for heartwood, the difference varying with the species.

Madison, Wis.

THE PARTICLE SIZE AND CONSTITUTION OF COLLOIDAL FERRIC OXIDE. I¹

BY J. B. NICHOLS, ELMER O. KRAEMER, AND E. D. BAILEY

It is generally recognized that an adequate interpretation of the properties and behavior of a colloidal solution is impossible without accurate information concerning the particle sizes of the dispersed material. Furthermore, the processes by which a colloidal solution may be formed can not be completely understood unless the changes in particle size during the successive stages of formation can be quantitatively followed.

Up to the present time, methods for measuring particle size have generally been limited in applicability, uncertain in accuracy, and often quite useless for quantitatively determining the non-uniformity in particle size. This has been particularly true for the colloidal solutions in which particle size approaches atomic dimensions and the behavior peculiar to the colloidal state is most pronounced. The invention and development of the ultracentrifuge during recent years by Svedberg and his associates² has provided a group of methods for determining particle size which promises to avoid many of the weaknesses of the earlier methods and to constitute the most powerful and most generally applicable technic for investigating dispersity in colloidal solutions. In Svedberg's laboratory attention has been focussed primarily on colloidal solutions of organic macromolecular materials, especially the proteins. With the exception of Rinde's work on colloidal gold,³ no detailed ultracentrifugal investigations of inorganic colloids have yet been published.

There can be no doubt that ultracentrifugal methods will be as effective for the inorganic colloids as for the organic ones. The most attractive group, of inorganic colloids for ultracentrifugal investigation is perhaps that of the so-called hydrous oxides, which include the oxides and hydroxides of beryllium, aluminum, silicon, vanadium, chromium, manganese, iron, cobalt, nickel, and most of the higher elements in the same groups of the periodic table. The hydrous oxides have been the subject of practically continuous study since the pioneering work of Graham. During the last few years they have been even more extensively investigated.⁴ On account of the dearth of reliable data concerning particle size and the wealth of data available on other aspects, ultracentrifugal analysis of the hydrous oxides should be particularly useful.

For initiating research in this field, we have selected colloidal ferric oxide. It has been studied as thoroughly as any other hydrous oxide; its formation by boiling a sufficiently dilute solution is relatively simple; its color offers advantages in connection with the ultracentrifugal analysis. The chemical composition of the dispersed phase in these colloidal solutions is to a consider-

¹ Communication No. 65 from the Experimental Station of the E. I. duPont de Nemours and Company.

² Svedberg: "Colloid Chemistry," 2nd ed. (1928); Kolloid-Z., 51, 10 (1930).

³ Rinde: "The Distribution of the Sizes of Particles in Gold Sols," Diss., Upsala (1928).

⁴ Symposium on oxyhydrates, Z. anorg. Chem., 42, 595, 885 (1929); Weiser: "The Hydrous Oxides" (1926).

able extent uncertain. In part, at least, it is sometimes a basic salt, sometimes a hydroxide, sometimes anhydrous ferric oxide. In this paper we shall use the term colloidal ferric oxide or hydrous ferric oxide, without desiring to imply, however, that the particles are in all cases simply Fe_2O_3 carrying adsorbed materials.

The introductory investigation, reported in this paper, dealt with colloidal solutions formed on hydrolysis of boiling dilute ferric chloride solutions. The particle-size determinations involved analysis of both the sedimentation velocity and the sedimentation equilibrium in the ultracentrifuge. The effect on particle size of duration of boiling, concentration of ferric chloride solution, and reversal of charge was determined. To obtain better insight into the hydrolytic changes, chemical analysis of the intermicellar liquid was carried out. In continuation of the investigation, attention will be given to other methods for forming the colloidal ferric oxide, the effects of dialysis and of aging, flocculation and peptization, solvation, and other such topics susceptible to ultracentrifugal analysis.¹

Experimental

The low-speed type of ultracentrifuge used throughout this investigation is similar to that described by Svedberg and Heyroth² and yields a maximum centrifugal force about 10,000 times that of gravity. For the determination of the particle-size distribution curves of the ferric oxide the following modified form of Stokes' law was used:³

$$r = \sqrt{\frac{9 \eta \ln \left(\frac{x+a}{a} \right)}{2 (d_p - d_m) \omega^2 t}}$$

where r is the radius of the particle in cm., η the viscosity of the medium, d_p the density of the particle, d_m the density of the medium, ω the angular velocity ($= 2 \pi n/60$, where n is the centrifuge speed in r.p.m.), t the time of centrifuging in seconds, x the distance in cm. along the cell from the meniscus, and a the distance of the meniscus from the axis of rotation.

The micellar or particle weight is determined from the sedimentation equilibrium by the relation⁴

$$M = \frac{2RT \ln \frac{c_2}{c_1}}{(1 - V\rho) \omega^2 (x_2^2 - x_1^2)}$$

For spherical particles, $M = 4/3 \pi \cdot r^3 d_p N$, where M is the micellar weight, N is the Avogadro number, R the gas constant ($= 83.19 \times 10^6$), T the absolute temperature, V the partial specific volume of the substance, ρ the density of the solution, c_2 and c_1 the concentrations at the points x_2 and x_1 distant from the axis of rotation of the centrifuge.

¹ Cf. Nichols: Colloid Symposium Monograph, 6, 287 (1928).

² J. Am. Chem. Soc., 51, 550 (1929).

³ For the theory underlying the determination of distribution curves by means of the ultracentrifuge, see Svedberg and Rinde: J. Am. Chem. Soc., 46, 2681-85 (1924); Rinde: "The Distribution of the Sizes of Particles in Gold Sols," Diss., Upsala (1928); and Svedberg: "Colloid Chemistry," 171 (1928).

⁴ Svedberg and Fåhræus: J. Am. Chem. Soc., 48, 430; Svedberg and Nichols: 3081 (1926); Svedberg: Kolloid-Z., 51, 10 (1930).

Most of the ultracentrifuge determinations were made at a speed of 10,000 r.p.m., corresponding to a force about 5,000 times gravity. The two sedimentation-equilibrium runs were made at speeds of 1950 r.p.m. and 2800 r.p.m. to give a favorable range of concentration in the final equilibrium.

A preliminary value of 4.5 was used for the density of the ferric oxide particles, which represents the estimates of Wintgen¹ and of Dumanskii² and our own pycnometric data. This value is uncertain owing to the difficulty of making proper allowance for hydration in the determination of the concen-

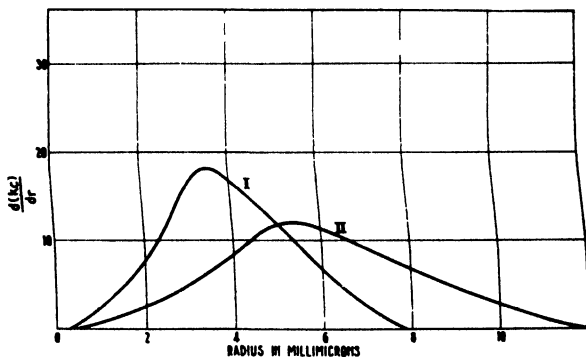


FIG. 1

Effect of the Density assumed on the Weight-Optical Distribution Curve of Ferric Oxide from 0.037 *M* FeCl₃ hydrolyzed One Hour at the Boil

Curve	Density	Mean Radius	Area
I	4.5	3.8 μ	64%
II	2.5	5.8	64

tration of colloid in the sol. It may therefore be necessary later to alter this value somewhat as a result of density determinations now in progress by procedures which take into consideration the hydration of the particles. To give an idea of the effect the assumed density will have on the distribution curve, Fig. 1 is presented. It represents the weight-optical distribution curve of the primary particles of sol Fe-30 prepared by the hydrolysis of 0.037 *M* FeCl₃ solution. The distribution curve corresponding to the real density will undoubtedly fall somewhere between the limits shown. Until more accurate data are available, however, the distribution curves for a density of 4.5 will serve as a satisfactory means for studying relative changes in the dispersity produced by various treatments.

The particle-size distribution curves obtained from the ultracentrifuge have been designated as *weight-optical* distribution curves because in a poly-disperse system of particles the light absorption may change with radius. Since, therefore, they ordinarily do not represent the true relation of weight of material to radius, the term "weight-optical" has been introduced to call attention to the fact that an apparent concentration is determined which is the product of the absorption constant *k* of the given radius by the concen-

¹ Kolloidchem. Beihefte, 7, 266 (1915).

² Kolloid-Z., 8, 232 (1910).

tration c of material of that radius. If the particles of the substance are fairly uniform in size, or if the light absorption is nearly constant over a range of sizes, then the weight-optical distribution curve will coincide with the weight-distribution curve.

Formation of the Colloid

Effect of Duration of Hydrolysis: A stock solution of approximately two-molar ferric chloride was prepared at the start of the work. The ferric oxide sols were made by diluting portions of this stock, adding the dilute FeCl_3 to boiling water, and hydrolyzing at 100° under a reflux condenser to prevent the loss of water and of hydrochloric acid formed during the hydrolysis. The hydrolytic action was stopped, after drawing off the sample, by cooling rapidly to room temperature.

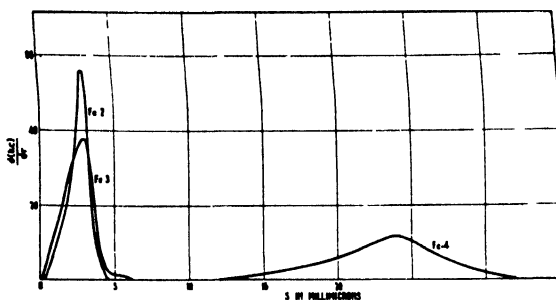


FIG. 2

Effect of Time of Hydrolysis on the Weight-Optical Distribution Curves of Ferric Oxide from FeCl_3

			Mean Radius	Area
(Fe-2)	16 min.	Hydrolysis of 0.005 M FeCl_3 at the boil	2.7 mμ	87%
(Fe-3)	60 "		2.6	94
(Fe-4)	24 hrs.		23.0	96

Fig. 2 gives the distribution curves obtained for the ferric oxide sols drawn off after sixteen minutes, one hour, and 24 hours of hydrolysis of 0.005 M FeCl_3 solution. The difference in area from 100% is due to non-centrifugible material. All samples were centrifuged without dilution. It is evident that there was very little growth in the particles between 16 minutes and one hour of hydrolysis, but by 24 hours' digestion there was a large growth, from 2.6 to 23 millimicrons mean radius. X-ray analysis of the colloid coagulated with potassium sulfate and dried at 105° showed a change from an essentially amorphous structure for the one-hour sample to a micro-crystalline hematite. The increase in particle size is therefore principally due to growth of the primary particles. Part of the increase, however, may result from flocculation, for x-ray analysis would not reveal the presence of some flocculated material accompanying the crystalline portion, and ultracentrifugal analysis would not distinguish between flocculated material and single crystals.

When a more concentrated solution was hydrolyzed there was such a rapid growth in particle size or agglomeration that, after eight hours' digestion,

most of the ferric oxide was in the form of a coarse, brick-red sediment. This sediment was too coarse to ultracentrifuge as a water dispersion; therefore, preparatory to the determination of particle size, it was washed and redispersed in 95% glycerin of viscosity roughly 250 times that of water. Fig. 3, giving the distribution curves for sols obtained after a one-hour and an eight-hour hydrolysis of 0.037 *M* FeCl_3 solution, shows a growth in mean size from 4.4 to 132 millimicrons. When these distribution curves are plotted on a more open scale, they resemble in form the other curves presented. An x-ray examination of the coarser, brick-red material from the eight-hour hydrolysis showed it

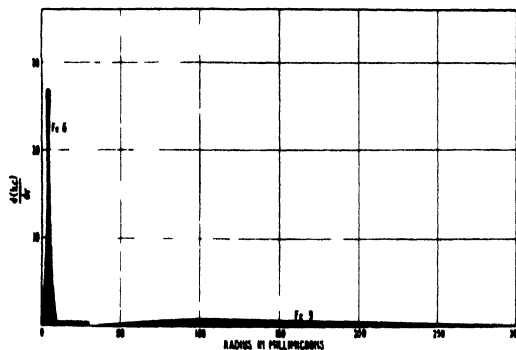


FIG. 3

Effect of Time of Hydrolysis on the Weight-Optical Distribution Curves of Ferric Oxide from 0.037 *M* FeCl_3

Sample	Time of Hydrolysis	Mean Radius	Area
(Fe-6)	1 hr	4.4 μ	90%
(Fe-9)	8 "	132	89

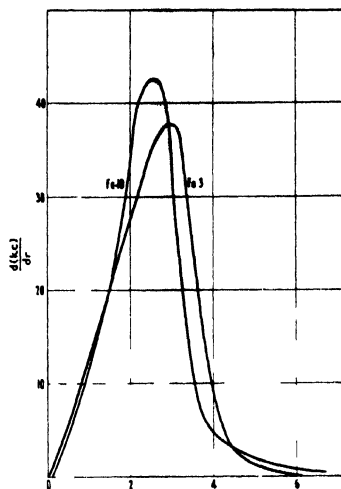


FIG. 4

Reproducibility of Ferric Oxide from 0.037 *M* FeCl_3 Hydrolyzed One Hour at the Bottom

	Mean Radius	Primary Area	Uncentrifuged Material
(Fe-3)	2.6 μ	89%	<4%
(Fe-10)	2.4	85	ca. 10

to be entirely hematite. The colloid formed by the one-hour hydrolysis also gave a more distinct x-ray diagram than was obtained by hydrolysis of the more dilute FeCl_3 solution; its composition was that of a hydrous ferric oxide, but the pattern has not as yet been identified. It is possible that it may represent an expanded lattice¹ of hematite or goethite.

The reproducibility of the hydrolytic process is demonstrated by Figs. 4 and 5. The second hydrolysis for each concentration was made approximately two months after the first and from the same stock ferric chloride solution. The agreement is reasonably good as is evident from the curves. In the more concentrated sols there always seems to be a small amount of coarser material which sediments too rapidly to permit the obtaining of more detailed information than simply the mean size under the experimental conditions required for proper investigation of the distribution of primary particle sizes. Accordingly this coarser, probably flocculated portion is indicated as a rectangle of the proper relative area at the right of the primary distribution curve.

¹ Simon and Schmidt: Zsigmondy Festschr. Kolloid-Z., 36, 55 (1925).

This coarser material undoubtedly would be the only portion visible under the ultramicroscope, because even highly diffracting gold particles of 4 millimicrons radius are scarcely visible under the ultramicroscope.

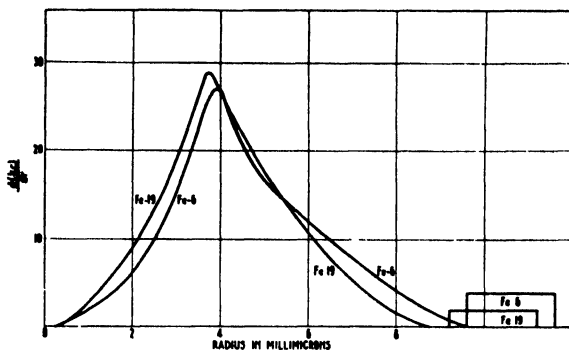


FIG. 5
Reproducibility of the Ferric Oxide from 0.037 M FeCl₃ hydrolyzed One Hour at the Boil

	Prim. M. Rad.	Prim. Area	Sec. M. Rad.	Sec. Area
(Fe-6)	4.4 mμ	90%	ca. 20 mμ	8%
(Fe-19)	4.05	95	—	4

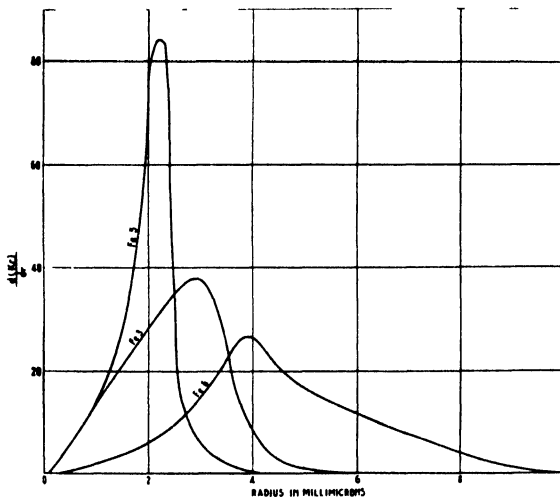


FIG. 6
Effect of Concentration during Hydrolysis on the Weight-Optical Distribution Curves of Ferric Oxide from FeCl₃

	Mean Radius	Area
(Fe-5) — 0.003 M	2.0 mμ	85%
(Fe-3) — 0.005 M	2.6	94
(Fe-6) — 0.037 M	4.4	90

FeCl₃ hydrolyzed one hour at the boil

Effect of Concentration of Ferric Chloride: The distribution curves (two of which have been shown above) of ferric oxide obtained from the one-hour hydrolysis of ferric chloride solutions, covering a 13-fold concentration range

up to 0.037 M $FeCl_3$, are combined in Fig. 6 to indicate the effect of concentration. Even with the assumption throughout of the same density of 4.5, there is only a two-fold increase in radius from 2.0 to 4.4 millimicrons, corresponding to about a ten-fold increase in weight. It is likely, however, that the sols prepared from the lower concentrations of ferric chloride also have a lower density and are therefore nearer to the mean size of the more concentrated sol than is indicated in the figure; that is, the apparent difference in distribution curves may in part be due to a difference in hydration or in swelling of porous particles.

The general inferences to be drawn from the curves presented in the last two sections are: First, the hydrolytic process is rapid and probably is com-

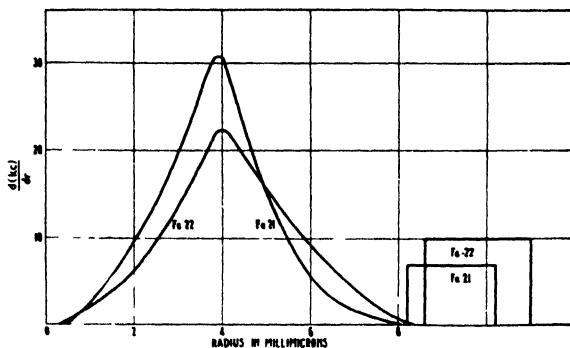


FIG. 7

Comparison of the Weight-Optical Distribution Curves of Ferric Oxide before and after Reversing the Charge with Potassium Citrate

	Charge	Prim. M. Rad.	Prim. Area	Sec. M. Rad.	Sec. Area
(Fe-21)	Positive	3.8 $m\mu$	84%	ca. 20 $m\mu$	14%
(Fe-22)	Negative	4.2	76	ca. 20	24

plete in the first hour; second, the succeeding digestion favors the secondary processes of dehydration, development of crystallinity, and growth of crystal size; third, the secondary aggregation and aging phenomena proceed more rapidly as the concentration of hydrolyzable material is raised.

Reversal of Charge: All of the sols described thus far have been the ordinary, positively charged sols stabilized with ferric or hydrogen ion. It is relatively easy, however, to reverse the charge with potassium citrate and obtain a negative sol stabilized with citrate ion. The resulting sol has much the same appearance as the original, positively charged sol, that is, a clear red color with only a faint muddy cast in reflected light. A fresh stock solution of ferric chloride was prepared and a solution of 0.04 M $FeCl_3$ was hydrolyzed at the boil. This sol was studied in the ultracentrifuge the following day and a portion of the sol was made 0.1 molar with respect to potassium citrate by adding molar potassium citrate to the original sol. The light absorption of the sol, now nine-tenths as concentrated as initially, was practically identical with that of the original sol, the increase in light absorption being due mostly to the production of greenish-yellow ferric citrate from the iron present in the intermicellar liquid.

Fig. 7 gives the distribution curves obtained for the two sols. The two curves are similar in form, the reversed sol having a slightly larger mean size of 4.2, as compared with 3.85 millimicrons for the original sol, and a greater amount of large, probably flocculated material of about 20 millimicrons radius. It seems likely that if the potassium citrate solution and the ferric oxide were mixed instantaneously and if the citrate concentration were adjusted more carefully then identical distribution curves could be obtained for a positive and a negative sol. If these two sols are mixed immediate flocculation of course occurs. On standing, there is a redistribution of ions resulting in a partial rezeptization.

Donnan Effect

The sols prepared from the hydrolysis of ferric chloride were studied in their original condition without any attempt to remove salts by dialysis both because a hydrolysis-aggregation would probably occur and because a Donnan

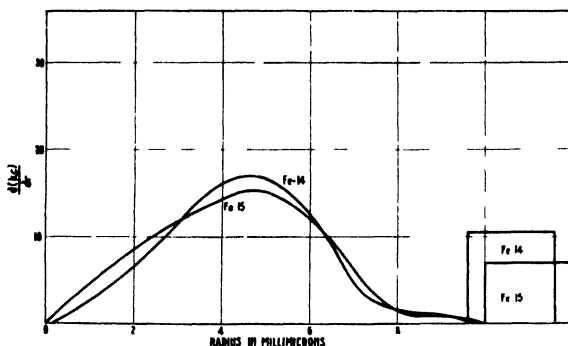


FIG. 8

Effect of Sodium Chloride on the Weight-Optical Distribution Curve of Aged Ferric Oxide from 0.037 M FeCl_3 hydrolyzed One Hour at the Boil

	Concn. NaCl	Prim. M. Rad.	Prim. Area	Sec. M Rad.	Sec. Area
(Fe-14)	None	4.6 $m\mu$	76%	ca. 19 $m\mu$	21%
(Fe-15)	0.1 M	4.2	77	ca. 19	14

potential might enter into the centrifuging of a purified sol arising from the partial separation of the large colloidal ions from small inorganic ions.¹ In order to make sure that enough salts were present in the sols to repress this possible electrical potential, molar sodium chloride solution free from sulfate was added to give final solutions 0.01 M or 0.1 M with respect to sodium chloride.

Fig. 8 gives a comparison of the distribution curves of an aged ferric oxide sol (from the first hydrolysis of 0.037 M FeCl_3) with and without the addition of sodium chloride. It is evident that there is no appreciable change in the distribution curve, but there appears to be a slight increase in secondary material of about 20 millimicrons in radius shown in the squares at the right. A similar study was made on the dilute sol prepared from 0.005 M FeCl_3 . If a Donnan effect were present the addition of sodium chloride would produce a shift in the distribution curve to larger particle sizes, but in the presence of

¹ Cf. Tiselius: Z. physik. Chem., 124, 457 (1926).

0.1 molar sodium chloride there was actually a slight decrease in mean radius to about 2.4 millimicrons; therefore, it is safe to conclude that all the sols contain sufficient electrolyte to repress any Donnan effect which might arise from the separation of charges.

Effects of Dilution

It is often mentioned that dilution of colloidal material produces a disaggregation, so an attempt was made to ascertain whether a colloidal ferric oxide would change in particle size on dilution.

Fig. 9 shows the distribution curve obtained for a two-months old sol (I from 0.037 *M* FeCl₃) compared with the curves for the same sol diluted to

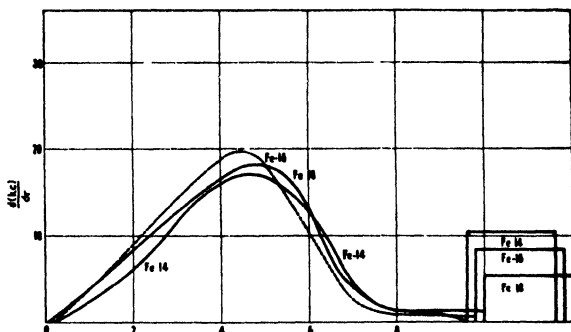


FIG. 9

Effect of Dilution on the Weight-Optical Distribution Curve of Aged Ferric Oxide from 0.037 *M* FeCl₃ hydrolyzed One Hour at the Boil

	Dilution	Prim. M. Rad.	Prim. Area	Sec. M. Rad.	Sec. Area
(Fe-14)	None	4.6 μ	76%	ca. 19 μ	21%
(Fe-16)	$\frac{1}{4}$ with water	4.2	81	16	17
(Fe-18)	$\frac{1}{2}$ with 0.05N HCl	4.45	82	ca. 20	11

one-quarter of the initial concentration with water (the dotted curve) or with 0.01 *N* hydrochloric acid, which is approximately the acidity of the intermicellar liquid. There seems to be no appreciable change when the sol is diluted with hydrochloric acid except a possible reeptization of some of the secondary material of about 20 millimicrons radius shown at the right of the curve. However, there seems to be a slight decrease in mean size from 4.6 to 4.2 millimicrons accompanying the dilution with water. It is possible that this change had not reached completion at the time of the centrifuging.

Sedimentation-Equilibrium Experiments

In order to eliminate trouble with a possible hindered diffusion of the larger particles of the sol and rather large diffusion for the smaller particles, sedimentation-equilibrium runs were made. The more dilute sols give abnormal sedimentation-velocity curves which, on inspection, seem to represent a range of very small, easily diffusible particles, and some material which exhibits hindered diffusion and sedimentation.

Figs. 10 and 11 and Tables I and II describe the final equilibria obtained for the two dilute sols prepared respectively from 0.003 *M* FeCl₃ and 0.005 *M*

FeCl_3 . In the same figures is also plotted the variation in apparent micellar weight with distance along the length of the cell. A mean micellar weight was calculated from the concentration gradient over the region of the cell in which the original concentration obtained at equilibrium. (This procedure was found permissible by comparing the sedimentation-equilibrium curves for known mixtures). The mean radius obtained from the mean micellar weight was larger in each case, 3.1 and 3.4 millimicrons as compared with 2.0 and 2.4 millimicrons from the sedimentation-velocity method, for the original,

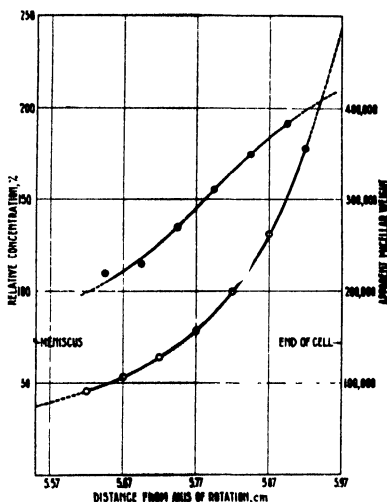


FIG. 10

Sedimentation-Equilibrium of Ferric Oxide from 0.003 M FeCl_3 hydrolyzed One Hour at the Boil (Fe-20)

- Variation of relative concentration with distance along the cell.
- Variation of apparent micellar weight with distance along the cell.

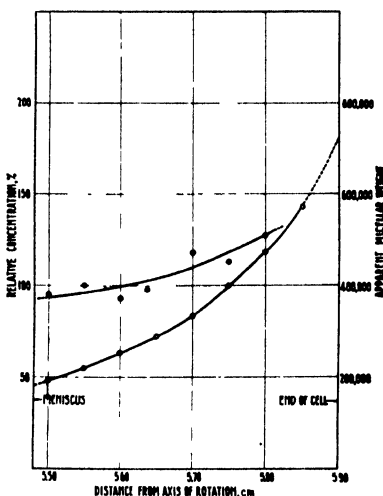


FIG. 11

Sedimentation-Equilibrium of Ferric Oxide from 0.005 M FeCl_3 hydrolyzed One Hour at the Boil (Fe-23)

- Variation of relative concentration with distance along the cell.
- Variation of apparent micellar weight with distance along the cell.

freshly hydrolyzed sols (Fe-5 and Fe-10). However, a redetermination of the mean particle size of the more concentrated sol, Fe-10, by the sedimentation-velocity method shortly after the completion of the sedimentation-equilibrium runs showed that the distribution curve had shifted slightly on aging from 2.4 to 2.8 millimicrons mean radius. Thus the discrepancy is not so large between the values obtained by the two methods. The residual difference between 2.8 and 3.4 millimicrons is very likely caused by the assumption of too high a density (4.5) for the particles. An analysis of the results from the two methods is being carried out to enable us to obtain a more reliable value of the density and the radius of the more or less hydrated particles in the sols.

Composition of the Intermicellar Liquid

The composition of the intermicellar liquid in a three-months old sol (Fe-30) obtained from the second hydrolysis of 0.037 M ferric chloride was investigated from several angles: namely, ultrafiltration, flocculation with potassium sulfate, and optical analysis of the non-centrifugible portion in the ultracentrifuge.

TABLE I

Sedimentation Equilibrium of Ferric Oxide (Fe-20)

0.003 M FeCl_3 hydrolyzed for one hour at the boil and diluted to one-half; speed 2800 r.p.m., ($\omega = 93.3 \pi$); mean centrifugal force, 4.950×10^5 dynes, distance of meniscus from center of rotation, 5.55 cm.; length of column, 0.42 cm.; thickness of cell, 0.8 cm.; density of Fe_2O_3 taken as 4.5, making $V = 0.222$ (prelim.); $T = 303.1^\circ$; photographic records taken 32.40.5, 49.5 hrs. after the start; Eastman Process plates, Eclipse developer, 2 min. development.

Distance from axis of rotation, cm.		No. of exposures	Mean relative concn., %		Micellar weight M
x_2	x_1		c_2	c_1	
5.92	5.87	5	177.1	131.2	383,900
5.87	5.82	10	131.2	100.0	350,000
5.82	5.77	10	100.0	78.7	311,200
5.77	5.72	10	78.7	64.1	270,600
5.72	5.67	10	64.1	53.8	230,300
5.67	5.62	10	53.8	45.7	219,100

TABLE II

Sedimentation Equilibrium of Ferric Oxide (Fe-23)

0.005 M FeCl_3 hydrolyzed one hour at the boil and diluted to one-half; speed 1950 r.p.m. ($\omega = 65 \pi$); mean centrifugal force, 2.373×10^5 dynes; distance of meniscus from center of rotation, 5.48 cm.; length of column, 0.42 cm.; thickness of cell, 0.8 cm.; same density and V assumed as in Table I; $T = 303.1^\circ$; photographic records taken 33.5, 39.5, 41.5, 48 hrs. after the start; plates and development same as in Table I.

Distance from axis of rotation, cm.		No. of exposures	Mean relative concn., %		Micellar weight M
x_2	x_1		c_2	c_1	
5.85	5.80	11	143.2	118.3	510,400
5.80	5.75	11	118.3	100.0	451,600
5.75	5.70	11	100.0	84.0	472,400
5.70	5.65	11	84.0	72.8	393,300
5.65	5.60	11	72.8	63.6	373,300
5.60	5.55	11	63.6	55.0	401,400
5.55	5.50	5	55.0	48.3	380,800

For the ultrafiltration a Giemsa ultrafilter¹ obtained from Carl Schleicher and Schüll Company was arranged so that a pressure of one atmosphere could be applied to the ferric oxide sol to force the liquid through the filter. Under the experimental conditions used about 15 cc. of ultrafiltrate an hour was obtained, the latter portion of which was slightly more colored than the earlier portions, probably resulting from the large increase in concentration of extremely small ferric oxide particles on the pressure side of the membrane towards the end of the filtration. In view of this increase in color the ultrafiltrate was sent through the filter again and a much lighter yellow filtrate was obtained.

¹ Biochem. Z., 132, 488 (1922).

Another portion of the sol was flocculated with an equal quantity of 0.0025 molar potassium sulfate, the precipitate centrifuged down to a compact mass, and the supernatant liquid poured off and saved for analysis and measurement of light absorption.

The only means available for determining the composition of the non-centrifugible material in the sol was the measurement of its absorption in the long-wave ultraviolet while the sol was being centrifuged. An attempt was made to collect a few drops of the liquid above the sedimented material at the end of a run in the ultracentrifuge, but the amount was too small to obtain an analysis on it. It should be possible, however, with a thicker cell to obtain enough liquid for a microanalysis of the chlorine content and perhaps a colorimetric analysis of the iron content.

The iron analyses were made by the Knop¹ dichromate method with diphenylamine as indicator, because neither the potassium permanganate titration nor the thiocyanate colorimetric method gave reliable results. Table III gives the analytical and optical data obtained for the intermicellar liquid. The analytical results obtained on the content of iron and chlorine in the ultrafiltrate and the supernatant liquid from the coagulation are expressed in terms of gram-equivalents per liter. The optical concentrations of the ultrafiltrate, of the supernatant liquid corrected to its original concentration before dilution with the coagulating potassium sulfate solution, and of the non-centrifugible material in the liquid above the sedimenting ferric oxide in the ultracentrifuge are expressed in terms of the per cent of parent ferric oxide sol possessing equal light absorption.

TABLE III

Composition of the Intermicellar Liquid of Aged Ferric Oxide Sol II (Fe-27)

Liquid	Analytical Concentration		Relative Light Absorption Equiv. to Fe ₂ O ₃ Concn.	
	Fe Concn. g.eq./l.	Cl Concn. g.eq./l.	366 mμ	435 mμ
Aged Fe ₂ O ₃ Sol II	0.1161	0.1123	100%	100%
Ultrafiltrate	0.0719	0.1066	31.5	6.5
Supernatant Liquid	0.0733	0.1125	31.7	6.5
Non-centrifugible	—	—	37.0	8.0

The analyses given in Table III show that the ultrafiltrate and the supernatant liquid from the coagulation have nearly the same iron content. As might be expected, however, the supernatant liquid contains more chlorine than the ultrafiltrate, owing to the displacement of chloride from the micelle upon the addition of the coagulating sulfate ion.² The chlorine content of the supernatant liquid corresponds very closely to the total chlorine present in the sol, thus indicating almost complete displacement of chlorine from the micelle at coagulation. The slightly lower content of iron in the ultrafiltrate undoubtedly results from a small adsorption of semi-colloidal ferric oxide on the ultrafilter.

¹ J. Am. Chem. Soc., **46**, 263 (1924).

² Cf. Linder and Picton: J. Chem. Soc., **87**, 1908 (1905); Weiser: J. Phys. Chem., **35**, 10 (1931).

The absorbing material present in the ultrafiltrate and in the supernatant liquid from the coagulation was practically identical, as measured by the light absorption both in the long-wave ultraviolet and in the blue region of the spectrum; both solutions possessed absorptions equivalent to practically the same per cent of that of the original ferric oxide sol. The ultraviolet light absorption of the ultrafiltrate was equivalent to one-half the concentration of ferric chloride employed in the preparation of the ferric oxide sol. On the other hand, analytical data showed 62% of the total iron to be present in the ultrafiltrate of this aged sol. These facts indicate that the iron compound present in the ultrafiltrate is less absorbing than that present in the sample of the six-months old stock ferric chloride solution freshly diluted to the concentration used for the hydrolysis. In other words, the freshly diluted ferric chloride contains a small amount of partly hydrolyzed iron. This portion would tend to be eliminated by re-solution in the excess of hydrochloric acid present during the aging of the ferric oxide sol.

As mentioned above, the third method of investigating the composition of the intermicellar liquid depended on the measurement of the ultraviolet light absorption of the solution in the meniscus region of the centrifuge cell after the sol had been centrifuged five hours at 10,000 r.p.m., a treatment which would remove colloidal material having a micellar weight greater than a few thousand from this region. The table shows that the relative light absorption of the remaining semi-colloidal, non-centrifugible material was somewhat greater than that of the supernatant liquid from the coagulation or of the ultrafiltrate. In the blue region of the spectrum the absorbing material of the meniscus solution is equivalent to 8% of that contained in the whole sol, as compared with 6.5% for the other two liquids, and in the ultraviolet it is equivalent to 37% of the whole sol, as compared with 31.5% for the other two liquids. The ratios are practically the same for the two wave lengths, but the magnitude is different because of different relative absorptions of ferric chloride and colloidal ferric oxide for the two wave lengths. The lower light absorption possessed by the ultrafiltrate may result from the adsorption of some semi-colloidal ferric oxide by the filter during ultrafiltration. Similarly, the equally low light absorption shown by the supernatant liquid may result from the removal by occlusion of some of this same material during coagulation with potassium sulfate. It would seem that the simple removal of colloidal material by centrifuging represents the mildest treatment of the system.

A general consideration of the ultracentrifugal, analytical, and light-absorption data indicates that ferric oxide sols formed by boiling dilute ferric chloride solutions contain unchanged ferric chloride, hydrochloric acid formed by the hydrolysis of the ferric chloride, and three principal colloid-fractions: a very highly dispersed fraction, which is non-centrifugible under the conditions of this investigation and which is perhaps a ferric hydroxyl chloride: the major fraction, having a particle size within the range of 1 to 10 millimicrons; and a coarser portion, which is probably formed by aggregation of the principal fraction. The light absorption of the principal fraction is very much greater than that of an equivalent amount of ferric chloride; the light ab-

sorption of the semi-colloidal material is intermediate in magnitude. The proportions of these constituents depend on the concentration of the initial ferric chloride, the duration of digestion, and the age of the sol. Prolonged heating is conducive to dehydration of the ferric hydroxide portion of the colloid-fractions, aggregation of the primary particles, and development of crystallinity. The hydrolytic process involves typical mass action effects; pH is a controlling factor in determining the amount of nascent $\text{Fe}(\text{OH})_3$ or $\text{Fe}(\text{OH})_2\text{Cl}$ which precipitates out to form the colloidal matter. During the aging which occurs at room temperature unpublished data indicate that re-solution of the colloidal ferric oxide to ferric chloride proceeds to an appreciable extent. The semi-colloidal fraction is probably most sensitive to this process. Greater initial concentration of ferric chloride, with the consequent production of more hydrochloric acid, causes this re-solution to proceed more rapidly and to a greater extent.

It is a pleasure to acknowledge the assistance given by two of our associates. The chemical analyses were made by Mr. E. S. Wilkins of our analytical department. The x-ray analyses were carried out by Dr. A. W. Kenney.

Summary

1. An ultracentrifugal study has been made of the particle-size distribution of undialyzed ferric oxide prepared by the hydrolysis of ferric chloride under different conditions.
2. It was found that the sols were reasonably reproducible, that dilution had no appreciable effect on the distribution curves, and that sufficient electrolyte was present in the undialyzed sols to eliminate any Donnan effect during centrifuging.
3. The hydrolytic process is rapid; subsequent digestion favors the secondary processes of dehydration and growth of crystal size.
4. X-ray analysis showed that the first particles formed in the hydrolysis were some form of hydrous ferric oxide which, on prolonged digestion, was converted into crystalline hematite.
5. The size of the primary particles (a few millimicrons in radius) produced in the first stages of the hydrolysis is not greatly influenced by the concentration of ferric chloride, but the rate of growth of the particles is much increased by higher concentrations of hydrolyzable material.
6. A sol reversed in charge by means of potassium citrate was found to have nearly the same distribution curve as the original, positively charged sol.
7. Sedimentation-equilibrium runs made on two sols gave approximately the same mean size as the sedimentation-velocity method.
8. Analytical and light-absorption data obtained on the intermicellar liquid indicate that the sol contains a semi-colloidal fraction approaching the dispersity of FeCl_3 in addition to the primary and secondary portions determined in the ultracentrifuge. The relative proportions depend on concentration, duration of digestion, and aging.

THE CONCENTRATION OF CATIONS IN CLAY SOLS*

BY RICHARD BRADFIELD

The application of certain inorganic salts to infertile soils often causes an increase in the growth of the crops. The failure of plants to make optimum growth on such soils is believed to be due to the fact that some essential ion is present in insufficient concentration to permit of its sufficiently rapid absorption for optimum growth. The response to fertilizer salts is commonly attributed to the fact that they increase the concentration of some essential ion at the disposal of the plant.

At first sight it would seem a simple matter for the chemist to examine an infertile soil in the laboratory and to make definite recommendations regarding its fertilizer requirements on the basis of these laboratory tests. Such attempts are usually far from satisfactory however. We have not yet been able to find a solvent for extracting the soil which resembles sufficiently closely the extracting power of the plant. We will consider here but one of the thousands of such attempts that have been made in the last century.

It has been found that the soil solution, i.e., the solution bathing the soil particles at water contents favorable for plant growth, can be displaced in apparently unaltered form by the use of the proper displacing technique. This displaced solution however frequently contains less of certain essential ions than is found necessary for good growth by solution culture studies in which the concentration of the cultural solution is maintained by continuous renewal. Such observations have raised the question as to the concentration of these essential ions on the surface of the soil particle. The relation between the plant root and the soil is a very intimate one. The plant root is known to give off considerable amounts of CO_2 which in contact with water would form H_2CO_3 which, in turn, could furnish both anion and cation for replacing other essential ions from the surface of the soil particle. The efficiency of such a mechanism would be undoubtedly influenced by the concentration of ions at the surface of the soil particles.

It has been clearly demonstrated¹ in the case of carefully purified hydrogen-clays that the concentration of hydrogen ions in a clay paste may be 1000 times as great as that in the ultrafiltrate from such a paste. It would seem probable from these results that the concentration of other cations might also be greater at the surface of the clay particles than in the intermicellar liquid. The results reported in this paper represent a preliminary effort to determine the order of magnitude of the concentrations encountered. It is planned to make a more exhaustive study of the problem.

*A contribution from Dept of Agronomy, Ohio Ag. Exper. Station and Dept. of Soils, Ohio State University.

¹ Bradfield J Phys Chem , 35, 364 (1931)

That the colloidal clay particles make an appreciable contribution to the conductivity of clay sols is shown by a comparison of the specific conductivity of a sodium-clay and its ultrafiltrate:

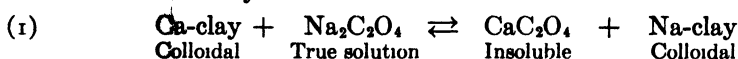
	$K \times 10^{-4}$
Na-Marion clay sol .022 N. 3% by wt.	2.37
Ultrafiltrate from above sol	1.00

That there is considerable conductance at the surface of colloidal clay particles has frequently been observed during the electrodialysis of clays. In a direct current field the clay particles are deposited on the anode membrane, apparently in a definitely oriented form. Finger-like projections are formed which if undisturbed will tend to bridge across the space between the membranes almost completely. These projections are frequently branched. Bubbles of gas can often be seen at their ends. If the current density has been high these deposits are usually rather dense and appear very much dehydrated when compared with a fresh deposit. If these bridging projections are broken by means of a stirring rod they fall to the bottom and until they have had an opportunity to become reoriented there is a very noticeable lowering in the amount of current thru the cell.

The following experiment illustrates the same phenomena. Two perforated platinum electrodes about 1 cm square and held rigidly about 2 cm. apart were dipped into a dilute H-Bentonite sol and 110 volts D.C. applied at the electrodes. In a few minutes practically all of the clay had collected on the anode and the space between the electrodes was almost completely bridged with an oriented clay deposit. In this condition a current of 220 milliamperes passed thru the system. The deposited Bentonite was then severed with a knife. The current fell immediately to 10 milliamperes. This would seem to indicate that when the clay particles are brought very close together, probably into contact, that there is considerable conductivity which is probably due to ions on the surface. If a film of water of sufficient thickness separates the particles this "ionic chain" is broken and the resistance increases.

The effect of concentration on the conductivity of a series of Miami-clay sols is shown in Fig. 1. These sols were prepared from an H-clay sol purified by prolonged electrodialysis by adding equivalent quantities of the different hydroxides. The maximum concentration secured was not great (3.0%) so that the particles were still separated from each other by relatively thick films of water. The conductivity of the Ba- and H-clays are almost identical, Ca-clay has about twice and the Na-clay five times the conductance of the Ba-clay. The pH values of the H-clay sols are also shown. (Fig. 1, Curve 1.)

An attempt was made to estimate the concentration of Ca and Ba ions in clays saturated with these ions by treating them with equivalent quantities of the soluble salts, sodium oxalate and sodium sulfate which tend to form by double decomposition insoluble salts with the cations of the clay. This reaction is rather unique in that only one of the 4 products of the reaction are soluble in the ordinary sense.



Some idea regarding the extent to which this reaction proceeds toward the right can be obtained from the change in the concentration of the $\text{Na}_2\text{C}_2\text{O}_4$. Ca-clay was treated with an amount of $\text{Na}_2\text{C}_2\text{O}_4$ exactly equivalent to the amount of Ca in the clay. The mixtures were allowed to stand with frequent shakings for over a week for equilibrium to be reached. An aliquot was then freed from clay by passing it thru an ultrafilter using a celloidion membrane. The first liquid passing thru the filter was discarded. The ultrafiltrate which is here considered as representing the intermicellar liquid was analyzed for the oxalate ion by titration with 0.01014 N KMnO_4 . In case of the samples treated with Na_2SO_4 the SO_4 in the ultrafiltrate was determined by conductometric titration with .0500 N barium acetate in 60% alcohol. Very satisfactory endpoints were obtained.

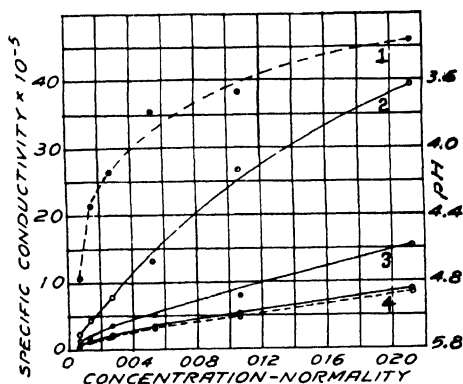


FIG. 1

The specific conductivity of Miami clay saturated with sodium (2), calcium (3), barium (4 solid) and hydrogen (4 dotted). The pH values of the H-clay are shown on curve 1.

TABLE I

The Reaction of Calcium and Barium Clays with Equivalent Quantities of Sodium Oxalate and Sodium Sulfate

Combination	Concentration Equivalents/liter	Specific Conductivity $\times 10^{-4}$	Milli- equivalents insoluble salt formed	Percent of total anion removal
1 Ca-Marion Clay + $\text{Na}_2\text{C}_2\text{O}_4$	0055	3.40	.28	51.0
2 Ba-Marion Clay + $\text{Na}_2\text{C}_2\text{O}_4$	0055	5.80	.13	23.6
3 Ba-Marion Clay + Na_2SO_4	0055	6.80	.20	36.3
4 Ca-Volclay Bentonite + $\text{Na}_2\text{C}_2\text{O}_4$.0046	3.47	.18	39.5
5 Ba-Volclay Bentonite + $\text{Na}_2\text{C}_2\text{O}_4$.0046	5.55	.00	0.0
6 Ba-Volclay Bentonite + Na_2SO_4	.0046	2.43	.28	61.2

A summary of the results obtained with two different clay sols is shown in Table I.

Under the conditions of this experiment 51% of the oxalate added was removed as CaC_2O_4 . This would seem to indicate that the concentration of Ca ions supplied by the clay was of the same order of magnitude as that supplied by CaC_2O_4 i.e., 4.24×10^{-5} equivalents per liter. The situation is complicated however by the fact that both substances on the right (Equation 1) are probably less ionized than the original compounds of the same cations. Evidence for this is found in the specific conductivities of the products concerned when present in concentrations of .0055 equivalents per liter or saturated in the case they are not soluble enough to reach this concentration.

TABLE II

Specific Conductivity of the Separate Compounds at 25° C

Substance	Concentration	$K \times 10^{-5}$
Na-Marion Clay	.0055 N	13.5
Ca-Marion Clay	.0055 N	5.4
Ba-Marion Clay	.0055 N	3.0
H-Marion Clay	.0055 N	3.0
$\text{Na}_2\text{C}_2\text{O}_4$.0055 N	61.0
Na_2SO_4	.0055 N	63.5
CaC_2O_4	Saturated	1.12
BaC_2O_4	Saturated	8.17
BaSO_4	Saturated	.28

While the conductivity of the Na-clay is over twice that of the Ca-clay it is only one-fourth that of the $\text{Na}_2\text{C}_2\text{O}_4$ or Na_2SO_4 . This would indicate a tendency for the reaction to proceed toward the right even though an insoluble salt of Ca were not formed. Numerous studies on the base exchange reactions of colloidal clays prove that this is true. The reaction does not proceed as far to the right however in case an insoluble salt of calcium is not formed.

The reaction:

$\text{Ba-Marion} + \text{Na}_2\text{C}_2\text{O}_4 \rightleftharpoons \text{BaC}_2\text{O}_4 + \text{Na-Clay}$ does not proceed as far as in the case of the calcium clay. This is due to (1) the fact that BaC_2O_4 is more soluble than CaC_2O_4 and (2) that Ba-clay is less ionized than Ca-clay. The two clays studied differ in their reactions. The amount of Ca^{++} replaced from Ca-Marion by $\text{Na}_2\text{C}_2\text{O}_4$ was greater than the amount of Ba^{++} replaced from Ba-Marion by Na_2SO_4 . The reverse was true with the bentonite.

The effect of the concentration of the sodium salt upon the progress of the reaction was studied by means of conductivity measurements. Ca-Marion containing .110 milliequivalents of calcium was treated with increments of $\text{Na}_2\text{C}_2\text{O}_4$ in the ratio shown in Table III. These samples were heated to 90° to facilitate the precipitation of the CaC_2O_4 then allowed to stand for a week. The conductivity was measured at $25.00 \pm .05^\circ \text{C}$. The values for the conductivity of the $\text{Na}_2\text{C}_2\text{O}_4$ alone were obtained by interpolation from the values in the International Critical Tables. The conductivity of the pure Na-Clay

and Ca-Clay was known. The above study indicates that about 50% of the Ca was replaced by Na when the ratio of $\text{Na}_2\text{C}_2\text{O}_4$ to Ca was 1. The conductivity curve indicates that the amount of replacement was directly proportional to the concentration of the $\text{Na}_2\text{C}_2\text{O}_4$. The correction due to the changing amounts of Ca- and Na-clays was calculated on the basis of these approximations. The error introduced is apparently quite small. The calcium was practically all removed from the clay by a 50% excess of $\text{Na}_2\text{C}_2\text{O}_4$ above that required for complete precipitation.

TABLE III

Determination of the Amount of Calcium displaced from a Ca-Marion Clay with Increments of $\text{Na}_2\text{C}_2\text{O}_4$ by Conductivity Method

No.	$\frac{\text{Na}_2\text{C}_2\text{O}_4}{\text{Ca}}$	Normality of $\text{Na}_2\text{C}_2\text{O}_4$	K of $\text{Na}_2\text{C}_2\text{O}_4$ $\times 10^{-5}$	K of Ca-clay + $\text{Na}_2\text{C}_2\text{O}_4$ $\times 10^{-5}$	K due to Na-clay + Ca-clay + $\text{CaC}_2\text{O}_4 \times 10^{-5}$
1	0	0	—	4.48	4.48
2	.25	.00275	31.5	16.50	5.9
3	.50	.0055	61.5	24.6	6.5
4	.75	.0082	90.0	34.4	7.1
5	1.00	.0110	119.0	46.8	7.8
6	1.25	.0137	145.0	63.0	8.9
7	1.50	.0165	172.0	73.2	9.6

No.	K due to $\text{Na}_2\text{C}_2\text{O}_4$ $\times 10^{-5}$	Normality of $\text{Na}_2\text{C}_2\text{O}_4$ left	Milli-equivalents of CaC_2O_4 formed	Percent of total Ca removed from clay
1	—	—	—	—
2	10.6	.0008	.020	18.1
3	18.1	.0015	.040	36.2
4	27.3	.0023	.059	53.6
5	39.0	.0034	.076	69.0
6	54.1	.0048	.089	81.0
7	63.6	.0057	.108	98.0

The results obtained in a similar study with a Ba-Marion clay treated with $\text{Na}_2\text{C}_2\text{O}_4$ are shown in Table IV. With a 50% excess of $\text{Na}_2\text{C}_2\text{O}_4$ only 54.6% of the Ba is replaced in comparison with 98% in the case of Ca. An interesting difference was noted in the degree of dispersion in the two series. The Ca-clay was highly dispersed with all additions above the 0.5 ratio. No decrease in dispersion was noted even in case of the 50% excess of $\text{Na}_2\text{C}_2\text{O}_4$. This substantiates other observations made in using $\text{Na}_2\text{C}_2\text{O}_4$ as a deflocculating agent in preparing soil samples for mechanical analyses. The amount of dispersion obtained seems to be independent of the concentration of $\text{Na}_2\text{C}_2\text{O}_4$ over a rather wide range. With the Ba-clay however complete flocculation of the clay resulted with all ratios of $\text{Na}_2\text{C}_2\text{O}_4$ to Ba of over 0.5. The higher

concentration of $\text{Na}_2\text{C}_2\text{O}_4$ required to replace the Ba of the clay lowered the charge on the particles more than the resulting replacement of Ba by Na on the particles increased it. MgC_2O_4 is even more soluble than BaC_2O_4 . These results indicate that while $\text{Na}_2\text{C}_2\text{O}_4$ is an excellent deflocculating agent for Ca-saturated soils that it might be of no value if the soil were saturated with bases which would tend to form even moderately soluble oxalates.

TABLE IV

Determination of the Amount of Barium displaced from a Ba-Marion Clay with Increments of $\text{Na}_2\text{C}_2\text{O}_4$ by Conductivity Method

No.	$\text{Na}_2\text{C}_2\text{O}_4$ Ca	Normality of $\text{Na}_2\text{C}_2\text{O}_4$	K of $\text{Na}_2\text{C}_2\text{O}_4$ $\times 10^{-5}$	K of Ba-clay + $\text{Na}_2\text{C}_2\text{O}_4$ $\times 10^{-5}$	K due to Ca-clay and BaC_2O_4 $\times 10^{-5}$
1	0	0	—	2.71	—
2	.25	.00275	31.5	24.3	11.8
3	.50	.0055	61.5	47.3	12.6
4	.75	.0082	90.0	70.0	13.5
5	1.00	.0110	119.0	96.2	14.2
6	1.25	.0137	145.0	116.8	15.1
7	1.50	.0165	172.0	129.6	15.9

No.	K due to $\text{Na}_2\text{C}_2\text{O}_4$ left in solution $\times 10^{-5}$	Normality of $\text{Na}_2\text{C}_2\text{O}_4$	Milli- equivalents of BaC_2O_4 formed	Percent of total Ba removed from clay
1	—	—	—	—
2	12.5	.0010	.0175	15.9
3	34.7	.0030	.0250	22.7
4	56.5	.0050	.032	29.0
5	82.0	.0075	.035	31.8
6	101.7	.0094	.043	39.0
7	113.7	.0105	.060	54.6

The reaction of Na-Clays with insoluble salts. The fact that only about 50 percent of the Ca was removed from a Ca-Clay by treatment with an equivalent amount of $\text{Na}_2\text{C}_2\text{O}_4$ suggests that a Na-Clay might form an appreciable amount of sodium oxalate if it were treated with the comparatively insoluble CaC_2O_4 . At first sight it may seem "rank heresy" to expect substances as insoluble as a Na-Clay and CaC_2O_4 to react to produce a substance as soluble as $\text{Na}_2\text{C}_2\text{O}_4$ in appreciable quantities. It must be remembered however that the other product of the metathesis is Ca-Clay which is much less ionized than Na-Clay. Ungerer¹ has studied this exchange reaction of Na-permutits and clays with certain insoluble phosphates and sulfates and found that with sufficient clay or permutit all of the insoluble phosphate or sulfate could be brought into solution. Reactions of this sort are worthy of

¹ Kolloid-Z., 48, 237 (1929); 52, 227 (1930).

further study because they may be of considerable economic significance. It is known as a result of practical field tests that on certain soils the application of different forms of insoluble phosphate fertilizers give just as satisfactory returns as the more soluble forms.

Na-Clay sols (100 cc.) were treated with 0.5 g. of the salts listed in Table V. The salts were prepared by treating the respective bases with a very slight excess of the acids. The precipitate was washed with distilled water by decantation using the centrifuge to accelerate the washings. The mixture was allowed to react for one week. The results shown in Table V indicate that the reaction did not proceed as far as in the experiments in which the clay sols were treated with a soluble salt. It is probable that equilibrium was ap-

TABLE V

The Reaction of Sodium Clays upon Certain Slightly Soluble Salts

	Salt 0.500 g. per 100 cc. of clay sol	Solubility Product	Na-Marion .0055 N 0.768 g. per 100 cc.	
			Millicquivalents of soluble salt formed per g. clay	Percent of Na of clay replaced
1	CaC_2O_4	2.0×10^{-1}	14	20.0
2	CaSO_4	6.1×10^{-1}	61	100.0
3	BaC_2O_4	1.7×10^{-7}	30	43.2
4	BaSO_4	1.0×10^{-10}	15	21.5

	Salt 0.500 g. per 100 cc. of clay sol.	Solubility Product	Na-Bentonite 0.046 N 0.600 g. per 100 cc.	
			Millicquivalents of soluble salt formed per g. clay	Percent of Na of clay replaced
1	CaC_2O_4	2.0×10^{-9}	.11	18.7
2	CaSO_4	6.1×10^{-5}	39	67.0
3	BaC_2O_4	1.7×10^{-7}	20	33.9
4	BaSO_4	1.0×10^{-10}	0.84	14.1

proached more closely in the case of these earlier experiments. In the case of the insoluble salts the concentration of all ions involved is very small. The reaction has proceeded far enough however to prove beyond question its significance. If 1 g. of a Na-Clay is capable of bringing into solution the SO_4 in 0.017 of BaSO_4 , one acre of soil containing 2,000,000 pounds of soil and 200,000 pounds of colloidal clay would on this basis render 3400 pounds of BaSO_4 soluble. Even if we consider the reaction in the field only 10 percent as effective as that in the laboratory we still obtain 340 pounds per acre, an amount which compares favorably with ordinary commercial fertilizer applications.

The reaction will probably not proceed so far if the soil is initially saturated with H^+ or Ca^{++} , the cations which predominate in the soils of the humid region. This point is receiving further study.

Further information regarding the concentration of Ca^{++} in a Ca-Bentonite was obtained by a study of the current-voltage curves obtained with the dropping mercury cathode in $\text{N}/1000$ CaCl_2 solutions and in a Ca-Bentonite sol containing the same total amount of exchangeable calcium. If we assume that at this dilution the CaCl_2 is completely dissociated the Ca-Bentonite appears to be only 11.2% dissociated or the concentration of Ca ions is 1.1×10^{-4} which is about one-fourth that of a saturated solution of CaC_2O_4 .

The fact that the reaction $\text{Ca-Clay} + \text{Na}_2\text{C}_2\text{O}_4 \rightleftharpoons \text{CaC}_2\text{O}_4 + \text{Na-Clay}$ proceeds only about half way even with the formation of the slightly dissociated Na-Clay favoring the reaction, indicates also that calcium ions are held even more rigidly to the clay particle, than they are to $\text{Ca}_2\text{C}_2\text{O}_4$.

Summary

1. The concentration of cations at the surface of colloidal clay particles is higher than in the intermicellar liquid in a carefully purified system.
2. The concentration of Ca and Ba ions in such purified clays is of about the same order of magnitude as in such insoluble salts as CaC_2O_4 and BaSO_4 .
3. Clays can decompose these very slightly soluble salts and liberate from them quantities of anions of the same order of magnitude as are supplied in ordinary fertilizer practice.

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THE COMPOSITION OF SOIL COLLOIDS IN RELATION TO SOIL CLASSIFICATION

BY HORACE G. BYERS AND M. S. ANDERSON

Introduction

For a period of approximately fifteen years investigators in the Bureau of Chemistry and Soils have been engaged in the accumulation of scientific data concerned with colloid material of the soil. During this period a large volume of accurate information has been secured which has been published in the form of bulletins and journal articles. So far as the Bureau publications are concerned, but little has appeared concerning theoretical aspects of the composition of the colloid. They have concerned themselves, for the most part, with methods of isolation and estimation, and determination of general physical and chemical characteristics.

Meanwhile it is recognized that two more or less clearly defined ideas concerning the character of the colloidal complex have been developed. The one which for some years was dominant, and which, without serious error, may be attributed to van Bemmelen and Stremme, regarded the soil complex as essentially a mixture of three oxides, those of silicon, aluminium and iron. The water and bases of the complex were regarded as held by 'surface' reactions which are not described in terms of the ordinary laws of chemical behavior. The other view, which, of course, is really much older, but submerged for a time by the flood of physico-chemical research, has reappeared, and, indeed, has again become orthodox, though as yet not fully formulated. This view regards the colloid complex as essentially a group of acids, organic and inorganic, which are both weak and unstable, as well as insoluble and essentially amorphous. The salts of these acids, which are the colloid, behave as described by the ordinary laws of chemistry modified by this unusual combination of properties. The essential complex is also in special cases modified by the possible presence of undecomposed minerals and of the ultimate products of their hydrolysis. The variants of this view are almost as numerous as the number of contributors to it. In the near future the Bureau of Chemistry and Soils expects to present a discussion of its accumulated data bearing upon this question. Some comments only will appear in the present paper.

In recent years, largely due to the activities of, or stimulated by, the Soil Survey, under the direction of Dr. C. F. Marbut, and based initially upon the investigations of Russian soil workers, there has been developed a system of soil classification dependent upon the properties of the soil profile. This classification takes into consideration, in addition to the dynamic factor of soil development, the parts played also by the parent material in the genesis of the soil. It is the purpose of the present discussion to consider some of our recently accumulated data in their relation to this system of classification and to the composition of the acid complex of the soil.

TABLE I

TABULAR ARRANGEMENTS OF SOIL GROUPS INTO CATEGORIES

Category VII	1. Pedalfers	2. Pedocals
Category VI	1. Podsollic soils 2. Lateritic soils	3. Pedocals of Temperate Zone 4. Pedocals of Tropical Zone
Category V	Sub-groups of Group 10 in Category IV	{ 1. Chernozem 2. Chestnut colored 3. Brown 4. Gray 5. Sub-groups of groups 9, 11 and 12 of Category IV, none of which have yet received dis- tinctive names
Category IV	1. Tundra 2. Podsoles 3. Gray-brown podsollic soils 4. Red soils 5. Yellow soils 6. Prairie soils 7. Laterites 8. Ferruginous laterites	9. Northern Temperate Pedocals 10. Mid-latitude Temperate Pedocals 11. Southern Temperate Pedocals 12. The various still unknown groups of Tropical Pedocals
Category III	1. Soils with perfectly developed profiles 2. Soils with imperfectly developed profiles	3. Soils with perfectly developed profiles 4. Soils with imperfectly developed profiles
Category II	1. Soil Series Groups (a very great number)	2. Soil Series Groups (a very great number)
Category I	1. Soil units based on texture of surface horizon	Soil units based on texture of surface horizon

Without making a critical historical résumé of the development, from a beginning made by Milton Whitney about forty years ago, of the system of soil classification used in the United States, it may be stated that, while it is the result of a field study of the characteristics of the soil, for many years it was largely confined to a study of the surface, and, to a lesser degree, of

the subsoil. The views of Ramann¹, based largely upon the data of Cushman^{2,3} and co-workers in the Bureau of Chemistry and the Office of Public Roads that the weathering of the silicates is "really a result of the hydrolytic action of water," has been accepted, and the work has been greatly influenced by the publication in 1914 of the work of the Russian soil scientists by Glinka.⁴ The work of the Soil Survey since 1890, influenced by these results and others, has resulted in the system outlined by Marbut,⁵ to be published in the near future. In this system, which was developed through a study of the soils themselves, the influence of environmental factors in producing, and in furnishing explanation of the observed results has been recognized. These factors are chiefly the vegetative conditions which are at the same time determining factors of, and a result of, soil and climate; the climatic conditions, especially of temperature and rainfall; the duration of the soil forming processes; the relation of the terrain to the drainage and to the water table, and the material producing the soil. In this classification, given in Table I, the whole soil profile is considered, Horizon A and its subdivisions, with Horizon B and its subdivisions together constituting what may be considered the solum, or true soil, while the disintegrated parent material is called Horizon C. It is of very considerable interest to discover whether this scheme of classification is reflected by the composition of the soil colloid.

Data and Discussion

Previous to 1924 only a very few chemical analyses of colloid material were available. These were, for the most part, either partial analyses of fine soil fractions or of clays, or were not associated with sufficient field information to permit of any general conclusions.

In 1924 a bulletin by Robinson and Holmes⁶ gave the analyses of 44 colloids derived from 10 soil series. On the basis of these analyses the authors drew some very significant conclusions regarding not only the constitution of the soil colloid itself, but also that the molecular ratio of silica to the sesquioxides in a colloid is characteristic of the soil series from which it is derived. They were led to conclude that rainfall is a very important factor in determining colloid composition in that silica is more readily removed by leaching than are the sesquioxides, and high rainfall tends towards the decrease of the ratio. Also since calcium and sodium disappear from soils more readily through leaching than do sesquioxides, therefore the molecular relation of the sum of these bases to the sum of the sesquioxides indicates, by its magnitude, the extent to which leaching has occurred. It follows that, in a general way, these ratios are parallel. In this bulletin, also, the authors call attention

¹ Ramann. "Bodenkunde" (1911)

² A. S. Cushman. The Effect of Water on Rock Powders. U.S.D.A. Bureau of Chemistry, Bulletin 92 (1905).

³ A. S. Cushman and P. Hubbard. The Decomposition of Feldspars. U.S.D.A. Office of Public Roads, Bulletin 28 (1907).

⁴ "Die Typen der Bodenbildung" (1914).

⁵ "The Soils of the United States" (1931).

⁶ "The Chemical Composition of Soil Colloids," U.S.D.A. Bulletin No. 1311 (1924).

to the relationship between the quantities of silica, alumina and iron oxide required to form the compounds kaolinite and nontronite on the assumption that these exist in the colloid and have the general formula $2\text{H}_2\text{O} : \text{M}_2\text{O}_3 : 2\text{SO}_2$.

Many other investigators have discussed the relation of silica to sesquioxides and of silica to alumina in their relation to the different portions of the soil profile, both in the soil itself and in the colloid fraction. Recently, G. W. Robinson¹ has called attention to variations of the magnitude of the silica-sesquioxide ratio as a result of profile development in Wales. He finds a general tendency toward increase of sesquioxides in the B horizon as compared with the surface soil. These results are in accord with those of Tamm² in Sweden, and, indeed, with all investigations of northern humid soils.

The extensive data now available for American soils, most of which have found publication in recent bulletins,⁴ render possible a general comparison between the soils and the soil-making processes, and for this purpose the data are presented in full in Tables II and III. In these tables the analytical results have been recalculated in order to better bring out the points under discussion.

In Table II are given the data for soils from both of the main sub-divisions of soils, the Pedocals and the Pedalfers (Marbut's Category 7). The pedocals are represented by the Amarillo silt loam from Texas (Marbut's Category 4) and the Barnes silt loam from South Dakota (Marbut's Category 5). The Pedalfers are represented by three podsols, the Superior fine sandy loam from Wisconsin, the Beckett loam from Massachusetts, and the Emmet fine sandy loam from Michigan. The gray-brown podsollic soils are represented by the Miami silt loam, Chester loams and sandy loams, and Leonardtown silt loams, the mean values of the data for which are given in Table II and the detailed data in Table III. The red soils are represented by the Davidson clay loam from North Carolina, and by the mean values of the Cecil clay loams and sandy clays from Virginia, North Carolina and Georgia, given in Table III. The laterites are represented by the Nipe clay from Cuba, which is a ferruginous laterite. So far as we know, there are no modern truly laterite soils in the United States. The prairie soils are represented by the Marshall silt loam from Nebraska and the Shelby silt loam from Missouri.

In the following discussion the soil making process, so far as its chemical relations are concerned, is considered as essentially one of progressive hydrolysis of the soil minerals. Out of this material the soil development processes, such as translocation or elimination of the products of hydrolytic action and other processes, produce the soil. Translocation of material involves true solution or colloidal suspension, or both. It is recognized, of course, that the hydrolytic process is profoundly influenced by the "catalytic" effect of the presence of carbonic and organic acids and its rate is also a function of the temperature and of the character of the material being acted upon. It is

¹ J. Agr. Sci., 20, 618-39 (1930)

² Meddel. Statens Skogsforsoksanst (Sweden) 17, 49-300 (1920).

³ Robinson and Holmes: Loc. cit; Holmes: J. Agr. Research, 36, 459-70 (1930); Holmes and Edington: U.S.D.A. Tech. Bull., 229 (1930); Denison: J. Agr. Research, 40, 469-83 (1930); Anderson and Byers: U.S.D.A. Tech. Bull., 229 (1931).

TABLE II
Chemical Constituents of Colloids from Soils characteristic of Various Soil Groups

Location of Profile			Horizon			Depth inches			Chemical composition calculated on the basis $\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 = 100$			Colloids of the Pedocal Group										Total base exchange capacity by BaCl_2 milli-equivalents
												Molecular ratios					Monovalent and divalent bases calculated as milliequivalents per 100 grams					
												SiO_2 per cent	Al_2O_3 per cent	Fe_2O_3 per cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$	$\frac{\text{Fe}_2\text{O}_3}{\text{Al}_2\text{O}_3}$	Ca^+ milli-equivalents	Mg milli-equivalents	K milli-equivalents	Na milli-equivalents	
✓ Amarillo, Tex.	1	0-5	62.09	27.09	10.82	3.10	3.88	15.19	255	52.8	103.2	56.9	1.9	220.8	56.6							
	2	10-20	62.19	27.42	10.39	3.09	3.85	15.84	243	56.7	132.0	53.9	0.3	242.9	62.8							
	3	30-40	62.43	27.28	10.29	3.13	3.87	16.06	241	65.4	138.9	53.1	1.9	259.3	63.4							
	4	54-64	61.35	28.84	9.81	2.97	3.60	16.57	217	61.7	140.4	51.4	3.2	256.7	69.2							
	5	70-75	62.20	28.56	9.24	3.06	3.68	17.79	207	75.0	125.5	38.9	1.9	241.3	65.6							
	6	96-100	62.97	27.57	9.46	3.18	3.86	17.66	219	56.4	142.4	48.0	0.3	247.1	70.0							
✓ Barnes, S. D.	A ₀	0-2½	62.65	23.47	13.88	3.30	4.53	11.93	379	59.2	47.6	44.8	58.1	209.7	—							
	B ₂	14-48	60.89	23.85	15.26	3.09	4.32	10.57	408	46.7	63.0	41.0	45.5	196.2	—							
	C ₂	60-78	64.00	21.48	14.52	3.55	5.05	10.31	432	—	—	—	—	—	—							
Colloids of Podsol Soils																						
Superior, Wis.	A ₀	0-3	66.44	23.89	9.67	3.76	4.71	18.12	260	62.1	52.1	16.8	8.4	139.4	78.1							
	A ₁	3-8	68.52	24.72	6.75	4.01	4.70	26.88	175	36.1	62.0	45.4	11.0	154.5	56.3							
	B	12-30	45.01	35.81	19.18	1.59	2.13	6.20	343	23.6	84.8	29.1	4.8	142.3	44.0							
Beckett, Mass.	C	30-40	55.14	28.77	16.09	2.40	3.24	9.08	357	21.8	169.2	26.1	1.9	219.0	39.9							
	A ₀	0-6	52.35	29.64	18.01	2.16	2.99	7.68	389	23.2	15.4	10.4	8.1	57.1	78.9							
	A ₁	6-11	59.80	31.56	8.64	2.74	3.22	18.30	175	14.3	51.1	53.3	7.7	126.4	51.1							
	B ₁	11-13	28.03	25.15	46.82	0.86	1.88	1.58	189	11.4	32.2	17.6	3.9	65.1	—							
	B ₂	13-24	39.46	36.71	23.83	1.28	1.83	4.39	416	5.7	58.5	42.0	9.0	115.2	53.7							
Emmet, Mich.	C	24-36	46.71	37.50	15.79	1.67	2.11	7.83	270	6.1	104.2	87.0	8.1	205.1	18.9							
	A ₁	1-4	61.13	29.26	9.61	2.94	3.55	16.88	211	19.2	8.9	55.4	23.9	107.4	—							
	B ₂	24-33	55.33	27.35	17.32	2.45	3.41	8.46	404	66.0	81.8	56.1	23.2	227.1	—							
	C ₂	48-60	62.50	19.92	17.58	3.40	5.33	9.41	566	—	—	—	—	—	—							
Colloids of Gray-brown Podsol Soils																						
✓ Miami Mean values of 9 profiles	A ₁	58.90	30.01	11.08	2.69	3.33	14.18	237	—	27.0	103.5	56.6	7.6	194.7	32.7							
	B	56.76	29.70	13.54	2.50	3.25	11.13	201	—	26.3	130.0	72.6	4.8	233.7	38.7							
	C	57.85	29.19	12.97	2.61	3.36	11.72	284	—	111.8	156.7	94.9	7.8	350.5	36.3							
	A	51.50	34.10	14.40	2.01	2.50	9.62	268	—	14.8	44.1	30.2	11.6	100.8	19.7							
	B	51.19	32.76	16.05	2.01	2.62	8.29	317	—	10.4	42.5	26.8	10.6	90.4	22.7							
	6 profiles																					

¹Where analyses show carbonates to be present an equivalent amount of Ca is deducted. In some cases, however, analyses do not include carbonate, which may be present.

TABLE II (Continued)

Chemical Constituents of Colloids from Soils characteristic of Various Soil Groups

Location of Profile	Horizon	Depth inches	Chemical composition calculated on the basis $\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 = 100$				Molecular ratios				Monovalent and divalent bases calculated as milliequivalents per 100 grams				Total base exchange capacity by BaCl_2 , milli-equiv- alents
			SiO_2 per cent	Al_2O_3 per cent	Fe_2O_3 per cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$	Ca^1 milli-equiv- alents	Mg milli-equiv- alents	K milli-equiv- alents	Na milli-equiv- alents	Total milli-equiv- alents		
Chester Mean values of 8 profiles	A	44-65	38.82	16.53	1.54	1.96	7.51	.273	20.5	77.5	20.9	3.5	122.4	25.6	
	B	46-94	35.39	17.42	1.69	2.26	7.36	.323	13.6	69.9	24.2	3.0	110.6	21.9	
	C	45-53	38.69	15.77	1.59	2.00	9.06	.271	11.8	46.8	19.0	3.0	80.6	15.3	
Davidson, N. C.	A	0-9	43.62	40.67	15.71	1.46	1.82	7.36	.247	20.7	45.6	10.6	1.0	87.9	18.3
	B ₁	9-36	43.63	37.43	18.94	1.49	1.97	6.10	.323	20.0	20.3	7.9	trace	48.2	12.6
	B ₂	36-60	41.41	34.46	24.13	1.42	2.03	4.54	.448	12.5	17.9	3.8	trace	34.2	15.8
	C	60+	41.44	34.92	23.64	1.40	2.01	4.64	.434	17.8	3.0	3.6	trace	24.4	15.6
Cecil, Mean values for 8 profiles	A	44-41	43.18	42.42	15.17	1.45	1.64	9.75	.184	7.5	22.0	15.0	1.6	45.3	11.6
	B	42-02	42.81	41.91	15.48	1.38	1.57	7.47	.228	6.0	15.1	8.6	1.9	34.1	8.1
	C	42-62	41.91			1.40	1.63	7.41	.238	4.8	14.5	10.3	2.1	31.7	7.7
Niipe, Cuba	1	0-12	11.51	17.89	70.60	0.31	1.09	0.43	2.525	8.2	2.5	trace	trace	10.7	3.1
	2	40-60	6.64	13.72	79.64	0.17	0.81	0.22	3.723	trace	3.5	1.5	0.6	5.6	2.0
	3	100-144	15.28	21.26	63.46	0.42	1.22	0.64	1.915	trace	10.4	3.4	1.0	14.8	2.1
Marshall	1	0-14	59.97	28.36	11.67	2.82	3.58	13.14	.263	42.5	99.7	47.3	6.8	196.3	—
	2	14-36	60.07	27.43	12.50	2.87	3.72	12.59	.292	48.5	80.3	44.0	4.8	177.6	—
Shelby	1	0-7	58.35	29.90	11.75	2.64	3.31	12.40	.252	47.1	92.7	25.5	7.1	172.4	—
	2	8-12	57.99	29.81	12.20	2.61	3.30	11.42	.262	46.0	92.8	24.0	6.8	169.6	—
	3	12-20	57.83	29.80	12.36	2.60	3.28	11.18	.265	48.9	100.2	26.8	9.3	175.9	—
	4	20-24	57.56	29.09	13.35	2.60	3.36	10.80	.294	58.2	107.7	28.0	12.3	206.2	—
	5	24-48	58.41	27.75	13.84	2.71	3.57	13.61	.319	77.4	102.7	34.6	14.2	228.9	—
	6	48-60	57.98	27.79	14.23	2.67	3.54	12.72	.328	83.8	102.7	35.2	13.2	234.9	—

¹ Where analyses show carbonates to be present an equivalent amount of Ca is deducted. In some cases, however, analyses do not include carbonate, which may be present.

TABLE III
Chemical Constituents of Colloids characteristic of Various Soil Series

Colloids of the Miami Series																					
Chemical composition calculated on the basis $\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 = 100$					Molecular ratios					Monovalent and divalent basis calculated as milliequivalents per 100 grams					Total base exchange capacity by BaCl_2 milli-equivalents						
Location of Profile	Horizon	Depth inches	SiO_2		Fe_2O_3		$\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$		SiO_2		Al_2O_3		Fe_2O_3			Ca	Mg	K	Na	Total	Total
			per cent	cent	per cent	cent	per cent	cent	per cent	cent	per cent	cent	per cent	cent							
Eaton Co., Mich.	A	6-10	58.48	30.01	11.51	2.65	3.29	13.46	.245					18.9	121.5	57.5	6.8	204.7	31.7	31.7	
	B	18-30	57.57	29.04	13.39	2.59	3.36	11.39	.295					28.9	153.3	76.8	8.1	267.1	37.5	37.5	
	C	34-40	58.29	28.12	13.59	2.68	3.51	11.36	.309					44.6	169.6	85.4	11.0	310.6	42.6	42.6	
Washtenaw Co., Mich.	A	0-8	59.45	29.76	10.79	2.75	3.39	14.58	.233					33.9	105.7	73.9	11.3	224.8	31.7	31.7	
	B	8-30	57.29	29.89	12.82	2.55	3.25	11.83	.275					33.2	136.9	97.2	8.4	275.7	33.8	33.8	
	C	54+	58.57	29.08	12.35	2.69	3.41	12.55	.271					183.7	161.2	100.4	10.6	455.9	23.7	23.7	
Branch Co., Mich.	A	2-8	60.47	29.84	9.69	2.84	3.43	16.52	.208					7.1	107.6	58.2	8.4	181.3	22.9	22.9	
	B	9-36	56.59	30.48	12.93	2.48	3.15	11.61	.271					23.5	136.4	90.9	3.2	254.0	33.7	33.7	
	C	40-60	58.15	29.75	12.10	2.62	3.31	12.72	.260					61.7	155.7	100.4	4.8	322.6	34.3	34.3	
Miami Co., Ind.	A	4-10	58.37	29.51	12.11	2.66	3.35	12.77	.263					32.8	109.6	55.4	6.8	204.6	29.3	29.3	
	B	12-20	56.81	29.08	14.11	2.52	3.31	10.66	.310					31.4	142.4	73.3	5.8	252.9	42.5	42.5	
	A	6-10	59.30	28.84	11.86	2.75	3.49	13.25	.263					34.3	97.7	55.0	4.2	191.2	31.3	31.3	
Blackford Co., Ind.	B	15-20	57.01	29.81	13.18	2.53	3.24	11.45	.283					31.4	123.0	60.7	4.5	219.6	40.3	40.3	
	C	40-50	57.35	29.57	13.08	2.55	3.28	11.62	.282					133.1	154.3	89.2	7.7	384.3	34.2	34.2	
Grant Co., Ind.	A	4-7	59.89	30.29	9.82	2.78	3.30	15.92	.207					28.9	120.5	64.3	8.1	221.8	36.8	36.8	
	B	11-18	56.44	30.37	13.19	2.48	3.15	11.33	.278					27.5	129.0	83.9	4.8	245.2	39.1	39.1	
	C	38-42	58.14	29.29	12.57	2.64	3.36	11.33	.274					81.0	142.9	102.5	6.5	332.9	32.8	32.8	
Hancock Co., Ind.	A	3-9	61.01	28.85	10.14	2.92	3.58	15.94	.225					32.1	107.1	51.0	6.5	196.7	32.9	32.9	
	B	10-26	56.58	29.60	13.82	2.37	3.23	10.84	.298					19.3	126.0	69.4	3.2	217.9	37.9	37.9	
	C	32-42	57.92	28.93	13.15	2.57	3.39	11.66	.290					52.1	147.8	101.1	9.4	310.4	35.2	35.2	
Hancock Co., Ind.	A	2-12	55.89	32.47	11.64	2.39	2.91	12.71	.229					32.8	58.0	52.7	11.0	154.5	36.5	36.5	
	B	16-32	55.83	29.54	14.63	2.43	3.30	10.10	.317					25.0	119.1	57.5	2.3	203.9	40.5	40.5	
	C	36+	56.90	29.28	13.98	2.53	3.30	10.89	.303					53.5	153.3	90.9	5.2	302.9	36.4	36.4	

TABLE III (Continued)
Chemical Constituents of Colloids characteristic of Various Soil Series

Location of Profile	Horizon	Depth inches	Chemical composition calculated on the basis $\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 = 100$			Molecular ratios			Monovalent and divalent basis calculated as milliequivalents per 100 grams					Total base exchange capacity by BaCl_2 milli-equiv- alents		
			SiO_2 per cent	Al_2O_3 per cent	Fe_2O_3 per cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$	$\frac{\text{Fe}_2\text{O}_3}{\text{Al}_2\text{O}_3}$	Ca milli-equiv- alents	Mg milli-equiv- alents	K milli-equiv- alents	Na milli-equiv- alents		Total milli-equiv- alents	
Rush Co., Ind	A	5-14	57.33	30.49	12.18	2.50	3.19	12.45	256	22.1	104.2	41.8	5.2	173.3	3	41.1
	B	14-18	56.75	29.48	13.77	2.51	3.27	10.92	299	16.8	103.7	43.7	3.2	167.4	43.3	
	C	60.64	57.45	29.48	13.07	2.57	3.30	11.64	283	28.5	168.7	89.2	6.8	550.1	51.2	
Mean Values	A		58.90	30.01	11.08	2.69	3.33	14.18	237	27.0	103.5	56.6	7.6	194.7	32.7	
	B		56.76	29.70	13.54	2.50	3.25	11.13	291	26.3	130.0	72.6	4.8	233.7	38.7	
	C		57.85	29.19	12.97	2.61	3.36	11.72	284	111.8	156.7	94.9	7.8	350.5	36.3	
Colloids of the Leonardtown Soil Series																
Prince Georges Co., Md.	A	0-7	51.51	33.24	15.25	2.03	2.62	8.95	293	20.4	52.5	31.0	12.9	116.8	—	
	B	7-17	50.86	33.04	16.10	1.99	2.61	8.36	312	14.3	52.5	27.8	11.6	106.2	—	
Prince Georges Co., Md	A	0-12	49.09	37.26	13.65	1.80	2.21	9.81	226	8.2	43.7	31.9	13.9	96.7	23.0	
	B	12-18	52.14	33.10	14.76	2.07	2.64	9.35	286	7.5	43.7	28.0	7.7	86.9	23.3	
Charles Co., Md.	A	0-7	51.97	33.33	14.70	2.05	2.27	9.36	282	16.8	44.1	28.5	13.9	103.3	21.0	
	B	7-14	51.61	32.58	15.81	2.05	2.69	8.64	311	12.9	33.7	22.3	11.9	80.8	21.5	
	A	0-7	51.28	33.83	14.89	2.03	2.57	9.11	281	16.1	38.7	28.7	17.4	100.9	18.5	
	B	7-14	50.72	33.12	16.16	1.98	2.53	7.24	305	8.6	37.7	22.1	16.8	85.2	21.5	
Prince Georges Co., Md	A	1-8	53.56	33.35	13.09	2.15	2.72	11.30	251	11.8	37.7	30.2	3.9	83.6	15.1	
	B	8-18	51.41	31.73	16.86	2.05	2.75	8.08	340	5.0	45.6	30.2	8.4	89.2	23.8	
	A	1-8	51.57	33.60	14.83	2.02	2.60	9.20	283	15.4	48.1	32.1	7.7	103.3	21.1	
	B	8-18	50.41	33.00	16.59	1.97	2.59	8.05	322	14.3	41.7	30.4	7.4	93.8	23.3	
Mean Values	A		51.50	34.10	14.40	2.01	2.50	9.62	268	14.8	44.1	30.2	11.6	100.8	19.7	
	B		51.19	32.76	16.05	2.02	2.62	8.29	317	10.4	42.5	26.8	10.6	90.4	22.7	

TABLE III (Continued)
Chemical Constituents of Colloids characteristic of Various Soil Series

Location of Profile	Horizon	Depth inches	Chemical composition calculated on the basis $\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 = 100$				Molecular ratios				Monovalent and divalent basis calculated as milliequivalents per 100 grams				Total base exchange capacity by BaCl_2 milliequiv- alents
			SiO_2		Al_2O_3		SiO_2		Al_2O_3		Ca		Mg		
			per cent	cent	per cent	cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$	$\frac{\text{Fe}_2\text{O}_3}{\text{Al}_2\text{O}_3}$	milli- equiv- alents	milli- equiv- alents	milli- equiv- alents	milli- equiv- alents		
Chester Co., Pa.	A	0-9	42.57	37.10	20.33	1.44	1.94	5.52	.352	30.7	140.9	20.0	5.5	197.1	29.9
	B	9-28	40.56	33.84	25.60	1.38	2.03	4.20	.483	12.1	111.6	20.2	1.9	145.8	18.4
	C	40+	43.13	33.46	23.41	1.51	2.19	4.86	.450	14.3	84.3	24.0	2.3	124.9	18.2
Cecil Co., Md.	A	1-8	42.47	39.74	17.79	1.41	1.81	6.32	.287	3.2	120.5	8.7	0.3	132.7	27.7
	B	8-30	47.88	33.96	18.16	1.78	2.39	6.97	.343	3.6	77.9	18.7	0.6	100.6	26.0
	C	42-60	46.96	42.92	10.12	1.61	1.85	12.25	.151	8.6	31.3	8.1	2.3	50.3	18.7
	A	0-9	46.67	40.52	12.81	1.65	1.95	9.63	.203	25.3	82.3	28.7	1.9	138.2	27.9
	B	9-28	46.27	38.53	15.20	1.62	2.03	8.06	.252	13.9	66.0	24.6	4.8	109.3	18.2
	C	28-50	47.35	41.64	11.01	1.64	1.93	11.38	.169	7.5	42.7	21.0	0.6	71.8	14.1
	A	0-7	48.30	37.39	14.31	1.76	2.19	8.92	.246	20.0	74.9	22.9	4.5	122.3	25.2
	B	7-32	50.36	33.25	16.39	1.98	2.57	8.13	.316	15.7	76.9	27.4	3.9	123.9	25.7
	C	44-60	50.36	40.68	8.96	1.84	2.10	14.86	.141	12.8	31.7	13.0	2.6	59.9	15.0
	A	0-10	41.22	43.57	15.21	1.31	1.60	7.16	.224	18.9	68.5	20.0	0.6	108.0	22.5
B	10-30	45.27	39.58	15.15	1.55	1.94	7.90	.245	12.5	60.0	20.2	0.6	93.3	19.9	
Harford Co., Md.	A	1-9	45.60	37.57	16.83	1.60	2.06	7.18	.287	18.6	63.5	22.5	2.9	107.5	19.9
	B	9-28	46.33	35.83	17.84	1.66	2.19	6.87	.319	11.4	34.7	21.7	1.9	69.7	18.1
	C	40-60	41.22	37.26	21.52	1.37	1.87	5.07	.370	11.1	42.2	20.6	5.8	79.7	12.0
Montgomery Co., Md.	A	0-8	41.81	37.32	20.87	1.40	1.90	5.30	.359	12.8	58.0	17.2	1.6	89.6	22.7
	B	8-34	46.60	33.53	19.87	1.77	2.35	6.22	.379	16.1	68.5	21.2	1.6	107.4	26.9
	C	54+	44.18	36.20	19.62	1.55	2.07	5.96	.347	16.8	48.6	27.2	4.2	96.8	13.5
Montgomery Co., Md.	A	0-8	48.59	37.36	14.05	1.77	2.20	10.01	.222	34.6	11.4	27.4	10.6	84.0	29.0
	B	8-32	52.27	34.61	13.12	1.79	2.56	10.54	.243	23.2	63.5	39.3	8.4	134.4	22.7
	A	44-65	38.82	16.53	1.54	1.96	7.51	.273		20.5	77.5	20.9	3.5	122.4	25.6
Mean Values	B	46.94	35.39	17.42	1.69	2.26	7.36	.323		13.6	69.9	24.2	3.0	110.6	21.9
	C	45.53	38.69	15.77	1.59	2.00	9.06	.271		11.8	46.8	19.0	3.0	80.6	15.3

TABLE III (Continued)
Chemical Constituents of Colloids characteristic of Various Soil Series

Location of Profile	Horizon	Depth inches	Chemical composition calculated on the basis $\text{SiO}_2 + \text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3 = 100$			Molecular ratios				Monovalent and divalent basis calculated as milliequivalents per 100 grams				Total base exchange capacity by BaCl_2 milli-equiv- alents		
			SiO_2 per cent	Al_2O_3 per cent	Fe_2O_3 per cent	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$	$\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$	$\frac{\text{Fe}_2\text{O}_3}{\text{Al}_2\text{O}_3}$	Ca milli-equiv- alents	Mg milli-equiv- alents	K milli-equiv- alents	Na milli-equiv- alents		Total milli-equiv- alents	
Colloids of the Cecil Series																
Goochland Co., Va	A	0-8	49.07	40.53	10.39	1.77	2.05	12.50	164	4.3	38.7	17.0	3.6	63.6	9.0	
	B	12-30	44.67	41.89	13.44	1.50	1.81	8.80	205	1.8	25.8	9.8	1.9	59.3	8.3	
	C	60+	44.53	39.66	15.81	1.51	1.90	7.45	255	2.9	27.8	8.9	1.6	41.2	7.6	
Rockingham Co., N. C.	A	4-10	44.71	41.34	13.94	1.51	1.83	8.48	216	7.1	—	21.9	0.6	—	8.0	
	B	16-38	44.66	41.49	13.85	1.50	1.82	8.54	213	5.4	11.9	13.6	0.6	31.5	6.9	
	C	70+	43.74	40.73	15.53	1.46	1.82	7.47	243	1.4	10.4	8.9	1.6	22.3	5.8	
Davie Co., N. C.	A	1-8	49.44	40.33	10.23	1.78	2.08	12.81	162	2.5	20.8	15.5	0.6	39.4	13.7	
	B	20-50	45.99	39.69	14.32	1.59	1.96	8.51	231	11.1	24.8	7.6	0.3	43.8	9.6	
	C	50+	45.39	39.38	15.23	1.55	1.95	7.89	247	11.1	25.3	21.3	0.6	58.3	10.5	
Rutherford Co., N. C.	A	0-5	41.78	44.59	13.63	1.33	1.59	8.12	196	7.9	27.8	7.4	1.9	45.0	9.4	
	B	5-36	38.26	44.18	17.56	1.18	1.47	5.77	254	8.9	6.5	3.8	4.2	23.4	5.6	
	C	72-96	36.10	50.13	13.77	1.03	1.21	6.94	174	8.2	6.9	3.8	2.9	21.8	7.0	
Clarke Co., Ga.	A	1-5	42.12	46.21	11.67	1.33	1.54	9.56	161	7.5	6.5	22.1	0.6	36.7	10.7	
	B	5-60	39.92	45.90	14.18	1.35	1.48	7.46	198	5.0	26.8	10.8	1.0	43.6	7.3	
	C	110-130	40.47	41.30	18.23	1.29	1.66	5.87	282	7.1	25.3	7.9	1.6	41.9	7.2	
Wilkes Co., Ga.	A	0-9	41.90	44.50	13.60	1.34	1.34	8.16	195	11.1	20.3	8.7	3.2	43.3	18.0	
	B	9-36	39.72	47.77	12.51	1.20	1.20	8.41	167	12.5	11.4	7.4	0.3	31.6	12.5	
Troup Co., Ga.	A	2-6	44.26	45.19	10.54	1.42	1.55	11.11	150	8.9	17.9	21.3	0.6	48.7	10.0	
	B	20-35	42.14	40.51	17.35	1.39	1.51	6.33	275	0.7	5.0	9.6	4.8	18.7	6.6	
	C	75-90	45.65	42.03	12.32	1.55	1.49	9.79	188	2.5	2.0	13.6	0.6	18.7	5.7	
Chambers Co., Ala.	A	0-7	41.97	42.71	15.32	1.12	1.12	7.24	230	10.4	22.3	6.4	1.6	40.7	14.0	
	B	8-24	40.81	41.08	18.11	1.31	1.31	5.96	283	2.9	8.9	6.4	2.3	20.5	8.1	
	C	25-59	42.43	40.11	17.46	1.40	1.40	6.43	279	0.7	3.5	7.9	5.5	17.6	10.3	
Mean Values	A	44.41	43.18	12.42	1.45	1.45	1.64	9.75	184	7.5	22.0	15.0	1.6	45.3	11.6	
	B	42.02	42.81	15.17	1.38	1.38	1.57	7.47	228	6.0	15.1	8.6	1.9	34.1	8.1	
	C	42.62	41.91	15.48	1.40	1.40	1.63	7.41	238	4.8	14.5	10.3	2.1	31.7	7.7	

also to be kept in mind that the consequences, as well as the degree of dispersion, are modified by the quantity of water entering into the operations, and by the character and quantity of the materials through which percolating waters must pass, as well as by erosional effects both at the surface and within the body of soil and soil material.

From Table II it will be noted that when the analytical data are recalculated so that the sum of the three chief constituents equals 100%, certain marked differences are evident in the different groups. In the pedocals the silica, alumina and iron oxide content is nearly constant. In the case of the Amarillo there are two distinct series of layers, 1 to 3 and 3 to 6. In each a layer is marked by the fundamentally characteristic accumulation of calcium carbonate. In the third stratum the quantity of CO_2 is 0.35%; in the fifth 11.82%.¹ In the soil itself the percentages were not determined. The field data, as collected by the Soil Survey indicate clearly that the lower set of strata represents an old and buried soil and, indeed, this fossil soil had reached a much higher degree of maturity than the present soil. The material from which both were developed is of the same character. These facts make the essential constancy of the colloid composition the more striking and illuminating. The maximum range of silica throughout the profile is 1.62%; of alumina, 1.75%; of iron-oxide, 1.58%. This constancy is also revealed by the molecular ratios of silica to alumina, a range between 3.60 and 3.88; of silica to iron oxide, between 15.19 and 16.57 for the solum, and in the whole profile between 15.19 and 17.99, and of silica sesquioxide between 2.97 and 3.18. This latter value is about fifty per cent greater than is required for the composition of kaolin.

The combined water also is strikingly constant. If we eliminate carbon dioxide loss and the loss due to organic matter and recalculate the data for the Amarillo profile, the range for the first four layers is 8.06, 7.36, 7.90 and 7.76. This constancy of the composition of the colloid of the various horizons is the more remarkable in view of the range of the mechanical composition of the soil.² The inorganic colloid content is 23.9% for the first horizon and 42.8% for the second. When we turn to the content of bases in the soil colloid the following relations appear. The magnesium content is high as compared with the other bases, and its constancy in the profile, except in the first horizon, leads to the suspicion of the presence of undecomposed minerals in the colloid, or of the existence of the same definite complex in all horizons, especially when considered along with the like constancy and high content of potassium. The high total base content in comparison with the total base exchange capacity and the total exchangeable base are in accord. The fact that in each horizon the excess of exchangeable base over the total base exchange capacity, as determined by the use of normal barium chloride, accords with the pH values of the whole soil. These values are, for the respective horizons, 6.5, 7.8, 7.8, 7.9 and 8.3.

¹ Anderson and Byers U S D A Tech Bull , 229 (1931).

² Anderson and Byers Loc cit

The same relations shown by the Amarillo profile are also shown by the Barnes profile with such exceptions as are to be expected from the facts that the Barnes soil is developed near the east side of the Chernozem area and therefore under higher rainfall and from calcareous glacial drift instead of calcareous sand and clay. There is a somewhat greater contrast between the C horizon and the A and B in respect to silica-alumina ratio and the silica-sesquioxide ratio. There is also shown by the distinct though slight change of silica alumina ratio, and the very marked alteration of the silica-iron ratio between the A and B horizon, definite indication of a concentration of alumina and of iron-oxide in the B horizon at the expense of the A, a process most marked in the podsol and podsollic soils of the humid regions.

The most interesting difference between these profiles is the difference between the calcium and magnesium content of the A horizon as compared with the B. In general, it may be said that the A horizon is enriched by the ash content of the plants, a part of which is derived from the B horizon. The Barnes profile, with its abundant organic matter, shows this relation for calcium and potassium and a marked decrease in magnesium content, while the Amarillo shows a slight decrease in calcium, and the expected decrease in magnesium and a very slight excess of potassium in the A horizon of the colloid as compared with the B horizon. This analytical difference in the potassium is very slight and probably not real, since the reverse relation is shown by the soil itself. The explanation of these differences is found in the greater rainfall and more luxuriant vegetation of the Barnes compared with the scanty rainfall and light vegetative cover of the Amarillo.

There is revealed in the data so far assembled no evidence of any free sesquioxide in the Amarillo colloid and of but small quantities of free oxide of iron in the Barnes.

The greater concentration of colloid in the B horizon of the Amarillo and the Barnes, as revealed by mechanical analysis, may either be due to colloidal-freshet-erosion from the A horizon into streams, or to eluviation from the A and concentration in the B horizon. If the latter, then the evidence points toward the transfer of the constituents as a whole, and to the existence of definite complexes, acidoids, of the silica with alumina and iron oxide.

The data given in Table II for the podsols offer some interesting contrasts. The podsols have two distinct portions of the A horizon, the surface layer high in organic matter and the highly leached "bleicherde." The A horizons are invariably high in silica and low in alumina and iron oxide, when the major inorganic constituents are alone considered, as compared with the B horizon. The silica-alumina ratio for the colloid of the Superior fine sandy loam is the highest yet noted in our analyses, and in the two other podsols is of the same order of magnitude as in chernozem colloids. The silica-iron ratio is exceedingly high, although quite appreciable quantities of iron compounds are present, both in the soil and colloid. That free hydrated iron oxide is not present in the A horizon is indicated not alone by this ratio but also by the color of the colloid and of the soil. By contrast in the B horizon, the silica sesquioxide ratio is low and in the Beckett profile reaches 0.86 in the B stratum, a value

approaching that of laterites. The silica-alumina ratio is, however, relatively much higher, the minimum being 1.83, which is close to that of true clay.

The silica-iron oxide relations are very illuminating. In every case this ratio is markedly different from that in the A horizon, the maximum variation being 18.30 in the A to 1.58 in the B. It is to be noted that in this comparison the total silica is considered. These facts and the color of the B horizon and its colloids leaves no element of doubt that in the podsol the B horizon represents a zone of enrichment at the expense of transportation of material from the A horizon and that the transportation, so far as segregation of materials is concerned, is almost, if not quite, wholly of hydrated oxide of iron. That this is true is also indicated by the relatively small increase in the percentages of colloid material in the A₁ and B₁ horizons. These are for the Becket from 3.9% to 5.8% and from the Superior 1.6 to 4.9. In this connection it may also be mentioned that the B₁ horizon is a zone of greater enrichment than is B₂, a fact which would seem to indicate that the concentration of the colloid is the result of a species of filtration in which suspended colloid, the dispersion or solution of which is favored by the organic matter in the surface, is flocculated, or precipitated, by the higher alkalinity of the subsoil, especially when it is young. (These relationships are not new but have been frequently referred to in studies of the whole soil). When the B horizon is once established as a zone of accumulation it becomes a more effective filter, or may even become impervious.

The quantity of bases present in the colloid of the podsol is notably less than in the chernozem soils and, except in the A₀ horizon, the base holding capacity is also somewhat smaller. In the A₀ horizon the high base holding capacity is increased by the presence of organic matter, this relation being characteristic of organic matter. The base exchange content of the podsol is notably less than in the chernozem and by consequence the degree of saturation. The greater quantity of calcium in the organic layer and the smaller quantity of magnesium occurs in all three soils. The increasing quantities of magnesium in the C horizon point to the presence of unhydrolyzed, or at least, less hydrolyzed minerals in the colloid of this horizon.

We may now turn to a consideration of the gray-brown podsol soils. We find in Table II the mean of eight profiles of Miami, six A and B horizons of the Leonardtown, and six profiles and two additional A and B horizons of the Chester soils. The details for each soil are given in Table III.

The Miami soils are developed from calcareous glacial drift under deciduous forest cover and under higher temperature and somewhat smaller rainfall than the podsol.

The Leonardtown is developed from the sandy material of the coastal plain and the Chester from gneisses and shale. The Leonardtown and Chester are developed at a much higher mean annual temperature than is the Miami, though also under forest cover, mainly deciduous.

In the Miami series the silica-sesquioxide ratio is much lower than in the pedocal soils and also very much lower than in the A horizons of the podsol, while it is much higher than in the podsol B₁ and B₂. On the other hand, the

silica-alumina ratio is of the same order of magnitude as that of the chernozems. The cause is evident from the silica-iron oxide ratio which is materially and invariably less in the B than in the A. (It is to be noted from the depth measurements that none of the A₀ horizons of the Miami samples were analyzed. These examinations were for a purpose other than that of the present considerations.)

Insofar as podsolization is regarded as a segregation or fractionation of colloidal material, it is evident that the effect is chiefly upon the iron oxide content of the colloids. That eluviation has occurred to a large extent is evident from the fact that in the Miami soils the colloid content of the B horizon¹ is invariably very much greater, from 50% to 400%, than in the A. It is recognized, of course, that this colloid deficiency in the A horizon is due in part to erosion (horizontal elutriation) but it must also be due to eluviation (percolation or perpendicular elutriation).

In the Miami soils the total bases in the B horizon are of the order of magnitude of the pedocals and are somewhat less in the A horizon. The calcium content is undoubtedly greater in the A₀ horizon (not shown in the tables) but the fact is indicated clearly by the complete analyses of Miami soils on file in the Bureau. The total base content of the Miami soils is greater than that of the pod sols and less than that of the chernozem, while the total base exchange capacity is less than either. These facts are indicative on the one hand of somewhat greater hydrolysis in the Miami and a considerably more effective leaching. Unfortunately, the base exchange content of these samples was not determined so that the degree of saturation is not available.

When we turn to the Leonardtown series we find a marked decrease in the silica sesquioxide ratio as compared with the series previously discussed, but the silica-alumina ratio is still well above 2 and is less in A horizon than in the B. The podsollic effect is most largely shown by the shifting of the iron oxide. The total base exchange content and the total base exchange capacity are both much less than in the Miami series. These facts are in accord with the general effect to be expected from the greater hydrolysis at higher temperature, and indicate extensive hydrolysis and the elimination of the freed bases and of silica. The strikingly small base exchange capacity in spite of the high silica alumina ratio also points to quartz particles in the colloid, a supposition in harmony with the very large content of silt in the soil which is upwards of 50%.² The most striking characteristic of this series is the uniformity of the colloid composition.

The mean values of the colloid from the Chester series are given in Table II. These include six complete profiles and two additional profiles of two horizons given in detail in Table III. In this soil the silica-sesquioxide ratio is well below 2, while the silica-alumina ratio is 1.96 in the A horizon and 2.26 in the B. This is in strong contrast with the reverse relations in the pod sols and Miami and the practical absence of such relation in the pedocals. The same difference is shown to a less degree in the Leonardtown series. There is

¹ Holmes and Edgington. U.S.D.A. Tech. Bull., 229, 7 (1933)

² Slater and Byers. U.S.D.A. Tech. Bull., 232, 18 (1931)

not in the Chester series so marked evidence of segregation of iron-oxide, though the increase of the silica-alumina ratio in the B horizon and the decrease of the silica-iron ratio being opposed indicate considerable differentiation. This differentiation is shown by the iron-oxide-alumina ratio which is considerably greater in the B than in the A, as is the case in all the podsoils and podsolized profiles. This differentiation in the B horizon is further emphasized by consideration of the mean value of the colloid content, which is 17.6% in the A horizon and 27.6% in the B.¹

The total bases of the Chester are of the same order of magnitude as those of the Leonardtown series. The mean values are slightly higher but this is due chiefly to one sample from Chester Co., Pennsylvania, in which the magnesium content in the A and B horizon is abnormally great. Mean values of the magnesium content and the greater content in the A horizon as compared with the B would seem to indicate the presence of some partially hydrolyzed magnesium silicate in the colloid. The low base exchange capacity of the colloid indicates a degree of leaching approaching that of the red soils given in the next group. It will be noted that the base exchange capacity of the A horizon is greater than that of the B, owing to the higher base exchange capacity of the organic matter.

Why a similar relation does not appear in the Leonardtown series is not clear since the mean percentages of the organic matter are 7.11% for the Chester (Holmes and Edgington: *Tech. Bull.*, **229**, 12) and 7.66% for the Leonardtown (Holmes: *J. Agr. Res.*, **36**, 464, (1928)). The explanation in the case of the Miami may be in the fact that the A₀ portion of those profiles, high in organic matter, was not analyzed. Nevertheless, in the A₁ and B in the Miami the organic matter mean values are 5.95% and 1.98% respectively. In these profiles the usual relation of higher base content in the A horizon obtains.

The red soils given in the next section of Table II are the Davidson and the Cecil. The data for the Cecil are the mean values for a series consisting of seven profiles of three horizons and one of only the A and B. The detailed data for these profiles are given in Table III.

The Cecil soils are derived from highly weathered gneiss or schists and the Davidson from diabase, basalt or other quartz free igneous material (Marbut). These two soil series may be described as lateritic, though not yet laterites. The colloid content of the Davidson soil is very high. As determined by the water vapor absorption method it is 27.3, 64.8, 66.5 and 20.6 for the respective horizons given in the table (Anderson and Byers: *Bull.*, **228**, 17). The mean values for the Cecils are 11.4 and 42.2% for the A and B horizons. In these soils the silica sesquioxide ratio lies well below the value of two in both soil series, and that of the silica-alumina in the Cecil is also much below two. In the Davidson soil the silica-alumina ratio is well below two in the A horizon and almost exactly two in the B₁, B₂ and C horizons.

Even if we assume that all the iron-oxide exists as free hydrate and that in these colloids there are no free quartz particles or free hydrated silica, it is

¹ Holmes and Edgington: *Bull.*, **229**, 8 (1930).

difficult to avoid the conclusion that free alumina, more or less hydrated, exists in these lateritic soils. In the B horizons the silica-iron ratio in both series is well below that of the A and indicates a distinct segregation by transfer of iron oxide to the B horizon to a greater extent than the corresponding transfer of alumina (podsolization). The same result is indicated by the iron oxide-alumina ratio. This podsolization process is more distinctly marked in the Davidson than in the Cecil profiles. It may be remarked parenthetically that podsolization is a species of natural fractionation of colloid material and that in the near future I. C. Brown of the Bureau will publish the results of his efforts to accomplish the same result by laboratory methods.

In these colloids great extent of leaching is indicated by the very low values of the base content and in particular that of calcium. The extreme degree of hydrolysis is indicated by the low base exchange capacity of the colloids. In both of these respects the weathering has proceeded much farther in the Cecil than in the Davidson soils. In view of the fact that we are dealing here with only one profile of the Davidson and with a very diverse set of Cecil profiles, the above general statement may seem over-bold, but it should be remembered that the work on the Miami, Chester, Leonardtown and Cecil soils indicates a very great degree of constancy in the colloid of a given soil series whatever may be the location of the individual sample provided only that the sample be a fair representative of the series.

When we come to a consideration of the only true laterite we have studied we find the process of hydrolysis and of leaching carried almost to the practical limit. This means the complete conversion of the silicates to alumina and iron oxide and the removal of the bases and also of silica by leaching. In the Nipe soil this is carried to practical completion, the silica-sesquioxide, silica-alumina and the silica-iron oxide ratios all falling to fractional values. Even in this soil the process of podsolization is still detectable in the relation between the silica and iron oxide and between the iron oxide and alumina. Also in this soil the higher value of the silica-alumina ratio, distorted as it is by the material being essentially an iron ore, indicates the reluctant yielding of the aluminosilicates to weathering, a fact also attested by the abundance of clay in the surface of the lithosphere. In this ferruginous laterite the total bases become extremely small and calcium is absent except in the surface layer. The total base holding capacity also becomes an almost vanishingly small quantity.

The Nipe represents, therefore, a soil that has completed its course and is essentially dead, a condition recognized by plants which, on this soil, are scanty and ill nourished. We have no corresponding soils in the United States, so far as the writers are aware. In the Bureau we have analyses of the colloid from a fossil aluminous laterite (Anderson and Byers: Bull., 229, 17) and of a deep layer from a similar material from Costa Rica (Anderson and Mattson: U.S.D.A. Bull., 1452, 2) in which the relations are of the same type. In the former the silica sesquioxide ratio is 0.84 and in the latter 0.55. They are not true soils, and are not included in Table II.

We may now, having traced the chemical relationships from West to East and from North to South, return to a consideration of the data contained in the last section of Table II. This section gives the composition of the colloid of the Marshall silt loam from Case County, Nebraska, recalculated from data found in U.S.D.A. Bulletin 1311 (Robinson and Holmes: "The Chemical Composition of Soil Colloids") and of the Shelby silt loam from Bethany, Missouri, recalculated from data soon to be published by C. S. Slater of the Bureau of Chemistry and Soils. These are prairie soils (see Marbut's classification, Table I) and are derived, according to Dr. Marbut, from loessial material in the case of the Marshall and from somewhat calcareous glacial drift in the case of the Shelby. In both soils the dominant vegetation is grass and the temperature and mean rainfall moderate.

The silica-sesquioxide, silica-alumina and silica-iron oxide ratios all show them to be closely related to the chernozem soils. The silica-alumina ratio indicates that hydrolysis has not reached the point where appreciable fractionation of the aluminous silicate has occurred by eluviation. The silica-iron oxide and iron oxide-alumina ratios indicate a certain but limited podsolization. That leaching has proceeded to a very limited degree is clearly indicated by the large values of the basic components and by the very slight concentration of calcium and total bases in the A horizon. The base exchange capacity and the degree of saturation of these colloids have not been accurately determined for the Marshall by methods comparable with those used for the other colloids. The values for the Shelby, however, show that both are essentially saturated soils. The lower horizons of the Shelby profile represent the composition of the glacial drift from which the solum is derived.

The mechanical analysis of the Shelby profile shows about 100% increase in the quantity of colloid in the lower horizons. As determined by the pipette method the percentages are 25.1, 40.6, 46.0, 37.9, 29.8, 31.0, 18.5 and 36.1%, and by the vapor absorption method 23.1, 49.6, 37.2, 27.0, 26.8, 16.5 and 30.6. In this case, then, as in the chernozem soils, the removal of the colloid from the A horizon may be ascribed to horizontal elutriation, erosion, or to eluviation. That the former is effective is evidenced by the character of the streams, especially in freshets, which traverse the prairie soils. That perpendicular erosion, eluviation, also occurs is evident from the data given. If so, it is clear that the transfer is of the colloid as a whole. The conclusion is clear that the colloidal complex in these soils is an essential unit, as contrasted with the colloid of the podsols and of the podsollic soils.

The essential characteristics of the prairie soils as represented by the Marshall and the Shelby series are those of the chernozem, modified by the absence of the zone of carbonate accumulation, due to adequate rainfall for percolation throughout the profile and by incipient podsolization. In these series there is but faint indication of laterization, but in the prairie region the soil surveys show the existence of soil series in which there is no doubt that examination will reveal the evidence of the active operation of this process. The chemical characteristics of the colloids of the prairie soils are in harmony with the high degree of fertility of these soils.

Summary and Conclusions

The striking differences shown by the colloids of the great soil groups given in Category 4 of Table I clearly reflect the field differences upon which the classification is based. The analyses show that the process of podsolization, fractionation of colloids, occurs wherever humid conditions are adequate to permit extensive leaching of the products of soil hydrolysis. They also show that the hydrolytic effects of water are greater, the higher the temperature. It is also clear that not only is the soil making process affected by moisture and temperature, but by the character and quantity of the vegetation upon the soil and that in turn the soil condition is reflected by the character and quantity of the vegetation it will support.

Two rather important inferences from differences between the colloids discussed may be drawn. When soils have but little colloid content it is well known that all attempts to build up a permanent store of available plant food are useless, since percolation rapidly removes the material not used practically at once. Yet such soils are not valueless, as many of the soils of Florida witness. It would appear from the data of Tables II and III that similar attempts with laterites and highly ferruginous soils are almost equally futile. Such soils must be "spoon fed." On the other hand, chernozem, prairie, podsol and podsolitic soils may, if exhausted of their exchangeable base content by over-cropping, be restored to their pristine productivity, provided they be not ruined by erosion, by the proper use of adequate fertilization, or perhaps even by the lapse of adequate time for non-exchangeable bases to become available, and that this renewal is, in a manner of speaking, a permanent restoration.

The other inference requires for its full substantiation a more elaborate discussion and fuller evidence than can be presented in this paper. It is, briefly, as follows: The progressive hydrolysis of the soil forming minerals results *primarily* in the production of an acid complex, probably polybasic, consisting of an alumino-silicic acid radical, in which silica-alumina ratio is greater than two, and in which, as a soil colloid, the acid hydrogen is partially replaced by bases. The salts of this acid, as well as the acid itself, are extremely slightly soluble in water. The details of the structural relations of this acid complex will vary with the structure of its parent material, and with the degree to which iron replaces aluminum in the mineral silicate. The existence of this complex in the colloids of the prairie and podsol soils is rendered extremely probable by the X-ray examinations carried out by Hendricks and Fry¹ and by subsequently obtained, unpublished data, on the Amarillo colloid. In these colloids the X-ray diffraction patterns are those of montmorillonite or ordovician bentonite, the latter term being taken to indicate the presence in the material of quartz. The presence of quartz thus indicated may be considered as due to primary quartz or to silicic acid freed as a result of hydrolysis. As hydrolysis proceeds the alumino-silicate is converted next to a complex having a silica-alumina ratio of two, and, since the iron compounds apparently are more easily hydrolyzable than are the corresponding alumino-

¹ Soil Science, 28, 457-479 (1930).

silicates, the colloid complex contains iron chiefly as the hydrated oxide. The hydrolytic influence being favored by time, high temperature and much moisture, eventually produces a complex consisting essentially of hydrated oxides and, with extensive percolation, eventually of those of iron and aluminum alone. At any stage of hydrolysis all of these various compounds may be present and the character of the colloids be dependent upon which stage of hydrolysis is dominant, and to what degree removal of products by water has proceeded.

This conception of the soil colloid necessarily envisages the possible presence, or, better perhaps, probable presence, of colloidal sized particles of quartz and of unhydrolyzed minerals in most colloids.

A soil colloid is, therefore, not to be regarded as a single complex, even if the inorganic portion be considered alone, but as a system not in equilibrium, but proceeding, at a rate determined by environmental conditions, from its birth in the rocks to its ultimate end, a dead soil—the laterite.

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WATER RELATIONSHIPS IN COLLOIDS

II. "Bound" Water in Colloids*

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There have developed in the last few years several methods by which "bound" water can be determined in a system containing colloids. There are nearly as many definitions of "bound" water as there are methods for determining it. All methods are consistent, however, in that they subject the total water in the system under consideration, to a set of conditions of one type or another which remove or otherwise change the state of a fraction of that water, leaving a portion unaccounted for, this being designated as "bound." The usual idea of "bound" water carries with it the picture of a portion of the water in a system as being associated with the colloid phase with such strength that it is no longer free to exhibit those properties which are characteristic of water, i.e., it is no longer available to act as a solvent, or it cannot be separated from the colloid by freezing or by subjecting the system to pressure, as in an ultrafilter. This water can, however, be removed readily by drying at 100°C. or under vacuum at ordinary temperatures.

Those who have worked with systems containing colloids have repeatedly found that the water contained in the system failed to respond in the usual manner to the conditions to which the system may have been subjected. This generally means that the properties of the water in the system have deviated quite markedly from those characteristic of dilute solutions. Such discrepancies may be explained upon the assumption that a fraction of the water has been removed from the normal state by some type of reaction with the non-water components present, thereby losing its colligative properties. The fraction of water calculated as acting in the normal manner is then designated as "free" water.

But the water thus calculated as "bound" water has not lost its colligative properties, as is evidenced by the fact that it can readily be removed from the system together with the remainder of the water present, when the sample is desiccated in a vacuum at ordinary temperatures. At equilibrium against an atmosphere containing no water vapor (relative vapor pressure equal to zero) all of the water originally contained in the system will be found to have disappeared. That the activity of the "bound" water could be zero when determined by its ability to act as a solvent and still possess a positive value of activity when determined by vapor pressure measurements is obviously a contradiction.

What, then, is "bound" water? Is it possible to give an explanation for these observed discrepancies which will place measurements of "bound" water in colloid systems upon a basis wherein the various methods may be compared and evaluated?

*Contribution from the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago.

Colloids belonging to the so-called "elastic" gel type give relative vapor pressure-water content curves which show a characteristic S-shape and which are truly reversible so long as no chemical change is brought about in the colloid. The following considerations are confined in their application to this group of colloids, which includes practically all biological colloids.

The relative vapor pressure-water content curve for such a colloid is as true a representation of the relationships existing between the colloid and its associated water as is the corresponding curve for a crystalloid. With the crystalloid this curve approximates closely that required by the laws of dilute solutions wherein equimolecular quantities of solute have the same effect upon the activity of a given amount of the solvent. But with the colloids, no approximation to the laws of dilute solutions is observed. In fact, the forces acting to reduce the activity of the water are not the same in the two cases. With the colloid, this is brought about, at least to a large extent, by the gravitational forces of partial valencies on the surface of the micellae, whereas in the dilute crystalloid solutions the effect is purely kinetic. The relative vapor pressure-water content curve for a colloid cannot be predicted from the laws of dilute solution, nor from any other data which are more easily or accurately attainable than the curve itself. Once having determined the curve for a given colloid, however, it becomes possible to predict the amount of water which will be associated with a given amount of that colloid in any system in which the activity of the water with which it is in equilibrium is known. Provided, of course, that the effects upon the activity of the solvent by the colloid and the crystalloid, or by the various colloid components, are the summation of the effects of the individual components when present alone at the same activity of the solvent, as is the case for crystalloid mixtures.

The object of the experiments described below is to show to what extent the above provision is a fact. Upon this as a basis, an interpretation of some of the various methods being used to determine "bound" water (i.e. the water associated with the colloid) will be made.

Newton and Gortner,¹ as a sequence to their finding that the development of winter hardness in wheat is accompanied by an increase in the hydrophilic colloid content of the plant, devised a method by which they could measure the "bound" water in plant saps. These investigators pictured this additional ability of the plant to withstand freezing as resulting from changes in the nature of the water which was present in the plant whereby it became less freezable. They argued that, if this were so, it was due to the influence of the colloid which served to remove the water from that condition in which it was freezable, i.e., the colloid had "bound" the water and destroyed its colligative properties. Such a change in the properties of the water should be detectable as a change in its ability to dissolve materials, such as sucrose, the fraction of the water associated with the colloid being considered no longer available to act as a solvent. They therefore sought to measure the amount of water which was thus changed, by measuring the difference between the observed freezing

¹ Bot. Gazette, 74, 442 (1922).

point depression of a sample of the expressed sap of the plant to which enough sucrose was added to make the solution molar in sucrose, and the calculated value of this freezing point depression if the total water in the sap had been available to dissolve sugar. From the difference in observed and calculated values could be calculated the amount of unavailable water, i.e., the water "bound" by the colloid. The definition which they give for "bound" water then, is that fraction of the water present in the system which is not available to act as a solvent for a crystalloid added to that system. They used a molar solution of sucrose, empirically, and gained very definite differences which were taken to indicate that the colloid was actually holding on to some of the water so tenaciously that the water could no longer act as a solvent for the sucrose.

Hill,² using a very accurate type of vapor pressure apparatus, has recently studied the "bound" water content of blood and muscles. His definition for bound and free water is much the same as that of Newton and Gortner, the "free" water present in a system being defined as "the weight of water in one gram of fluid or tissue which can dissolve substances added to it with a normal depression of the vapor pressure"; the definition of Newton and Gortner differs from that of Hill only in that they used "freezing point depression" in place of "vapor pressure depression." Hill's method may be outlined as follows. To determine the water bound by muscle colloids, for example, he weighs a muscle which has been thoroughly soaked and washed with an isotonic Ringer-Locke's solution and then places it in another solution which is twice as concentrated in all components as the original Ringer's solution. The amount of this second solution added to the muscle is such as to contain the same weight of water as is present in the muscle sample. If every non-aqueous component of the muscle possessed an H_2O activity- H_2O content curve which obeyed the laws of dilute solutions the activity depression of the water in the final mixture of muscle plus two-Ringer's solution should equal 1.50 times that in the muscle before bringing it into equilibrium with the final solution. Thus when it is found that the activity depression in the final equilibrium mixture is greater than 1.50 times that of the water in the original muscle before mixing with the two-Ringer's solution, this difference between theoretical and observed values is taken to indicate that a portion of the water in the muscle is bound by the colloid and is unavailable to act as a solvent for the crystalloids contained in the two-Ringer's solution which had been added.

Another and quite different method for measuring "bound" water, which was devised by Rubner³ and used by Thoenes,⁴ has been improved upon in the last few years by Robinson⁵ and used to determine the relation of "bound" water to winter hardiness in insects. This method consists, in outline, in freezing the specimen at a temperature of -20°C . and then, by calorimetric means, determining the amount of water which has frozen out under these

² Hill: Proc. Roy. Soc., 106A, 477 (1930).

³ Abhand. preuss. Akad. Wiss., No. 1, page 1 (1922).

⁴ Biochem. Z., 157, 174 (1925).

⁵ J. Econ. Entom., 20, 80 (1927); Colloid Symposium Monograph, 5, 199 (1927)

conditions. The difference between total and freezable (or "free") water is taken as a measure of the "bound" water in the system. Rubner was interested in the distribution of water in samples of blood and other tissues, and used the temperature, $-20^{\circ}\text{C}.$, as the freezing temperature because he considered that at that temperature all the water which was associated with the salt (NaCl) present in his samples would have been frozen out (although $-23^{\circ}\text{C}.$ is the point at which the eutectic mixture forms) and that any water left unfrozen could be attributed to the influence of the colloids present.

Some other methods for the "bound" water measurement will be mentioned briefly later.

Since we are primarily interested in the amount of water which will be associated with the colloids in a given mixed system, we will define "bound" water in terms of unit mass of the colloid with which it is associated. The definition for "bound" water in a system containing colloid and crystallloid components, then, will be that amount of water which is associated at equilibrium with a unit mass of the colloid component. The amount of water which will be found to be associated with a given colloid must vary with the relative vapor pressure or activity of the water. It therefore becomes necessary when speaking of the "bound" water content of any mixed system to specify the activity of the water in the system at which the "bound" water measurement is made.

Experimental

In working with biological systems, the chief difficulty in the measurement of "bound" water lies in the impossibility, from activity measurements alone, in distinguishing between the relative influences which the various components may be exerting upon the water present. A knowledge of the water activity-water content curve for each component separately is necessary for such a calculation. Then, knowing the activity of the water in the sample, and assuming no reaction to have taken place between the non-water components upon mixing, the water associated with the colloid can be found by reference to its specific curve. But this is not possible with systems which cannot be readily and accurately analysed into their components.

Newton and Gortner, and Hill, have done the next best thing, that of measuring the deviation, between two arbitrary points, of the H_2O activity- H_2O content curve for the system containing the colloid, from that curve which is calculated for the system had it been following the laws of dilute solutions between these points. The results, while making comparisons between samples possible, do not give true values of the total amount of water associated with the colloid at the activity of water at which the measurements are made. Nor do these methods intimate that the value for "bound" water so obtained would vary if the arbitrary points on the curve were changed.

However difficult it may be to get at the true values for "bound" water when working with a complex biological system, it is not difficult to test out the methods so far devised for its determination, to see whether or not they can be interpreted in terms of the theory above outlined. This can be done

by determining the relative vapor pressure-water content curve for a purified (salt-free) colloid and then finding whether or not the values for "bound" water as found by the various methods coincide with values forming points on this curve.

The relative vapor pressure-water content curves for various colloids were determined in a manner described in the first paper of this series wherein the isotenoscope method for measuring vapor pressures of liquids has been used for moist colloids with satisfactory results.

The methods of Newton and Gortner and of Robinson have been followed in making the measurements of "bound" water.

Method of Newton and Gortner

The colloids used in this group of experiments were gum arabic and sodium caseinate. The former was prepared for use by grinding up selected sorts. A sample of this colloid was later electro-dialysed free of ash and found to require 84×10^{-6} equivalents of alkali per gram to neutralize it. The colloid as used thus contained this amount of ionizable calcium and sodium. The sodium caseinate, upon which only one series of determinations was made, served as a check upon the results obtained with the gum arabic. It was prepared from ash-free casein by the addition of 55.5×10^{-6} equivalents of NaOH. This brought the pH of the sample to 6.9. The sample was then dried (not completely) in vacuo at $35^{\circ}\text{C}.$ and ground up. It contained no protein hydrolytic products.

In order to obtain their value for "bound" water, Newton and Gortner made use of three freezing point determinations. The first, Δ , was that of the original plant sap or colloid containing solution, the next was Δ_o , the freezing point of this solution after sufficient sucrose had been added to make a molar sucrose solution with the total water present, and the third was Δ_c , the freezing point depression of a molar solution of sucrose in absence of colloid or other non-water compound.

The initial depression, Δ , was taken to be due to the crystalloid components of the sample and was therefore subtracted from the value of Δ_o in order to eliminate the water held by such crystalloid from the water calculated as "bound" by the colloid. The equation they used to obtain the grams of water bound by the colloid per 1,000 grams of total water was

$$\text{grams} = \frac{(\Delta_o - \Delta) - \Delta_c}{\Delta_o - \Delta} \cdot B$$

where B was the number of grams of water in the molar sucrose solution which was considered free to mix with the remaining solutes in the solution. Sucrose shows an abnormally high molar freezing point depression ($-2.085^{\circ}\text{C}.$ in their experiments). The sucrose is supposed to be dissolved in the form of the hexahydrate, thus leaving only 892 grams of the total to act as solvent.

In the calculations made in this paper, B is taken as equal to 1000 in the above equation, because it is considered that the abnormal effect of the sucrose is

taken care of when Δ_o is subtracted from $(\Delta_o - \Delta)$. At any rate the results so obtained are more nearly in accord with those obtained when alcohol and other crystalloids having normal molar freezing point depressions are used in the place of sucrose in the experiment.

Since crystalloid-free colloids are used in the present experiments (crystalloid ions in salt combination with the colloid are considered as a part of the colloid in these experiments), the initial freezing point, Δ , of the colloid solution, besides being almost negligible, is really due to the colloid or its associated ions and should not be subtracted from Δ_o because it is desired to obtain values for the entire amount of water associated with the colloid fraction at the various activities of water in the solutions, in order to compare them directly with values obtained by the relative vapor pressure determinations on the colloid.

The equation for calculating the grams of water associated with one gram of dry colloid (per 1000 grams total water) then becomes

$$\text{grams} = \frac{(\Delta_o - \Delta) \cdot 1000}{\Delta_o \cdot A}$$

where A = grams of dry colloid per 1000 grams of water, Δ_o = observed freezing point depression of water in crystalloid-colloid solution, and Δ = freezing-point depression of crystalloid solution of same molar concentration but containing no colloid. (Equals Δ_s for sucrose and Δ_a for alcohol in following tables.) The activity of water can be calculated from the freezing point depression according to the following equation,⁶

$$\log a = -0.004211\Delta - 0.0000022\Delta^2$$

where Δ is the freezing point depression of the solution the activity, a , of which is desired.

First, a series of experiments was made in order to obtain a comprehensive picture of the effects of mixtures upon the activity of water, especially when one of the components of the mixture was a colloid. Sucrose or ethyl alcohol were used as the crystalloid components of the mixtures. Freezing point determinations were made by the usual procedure, using a Beckmann thermometer and a cooling bath of salt water and ice. The solutions were prepared by adding weighed amounts of the dry crystalloids to a weighed amount of doubly distilled water and then dissolving in this solution a weighed amount of air dry colloid, the water content of which was known. Freezing point determinations were made on this final mixture and upon a solution of the crystalloid in water of exactly the same concentration. The difference in these freezing point depressions was considered as due to the association of a fraction of the water present with the colloid and its associated ions in such a manner that, *at the activity of the water in the solution*, it was not available to act as a solvent for the crystalloid. This amount of water could be calculated as above described. The total water thus held by the colloid divided by the number of grams of air dry colloid present would give the value for the water

⁶ Lewis and Randall. "Thermodynamics," 284 (1923).

in the solution associated with one gram of air dry colloid. To calculate the amount of water associated with one gram of bone dry colloid it was only necessary to use the equation

$$X = (S/B \cdot 100) + C,$$

where X = grams of water bound by 1 gram of bone dry colloid, S = grams of water bound by 1 gram of air dry colloid, as calculated from freezing point depression data, B = bone dry weight of one gram of air dry colloid and C = weight of water in 1 gram of air dry colloid.

The water content of the air dry colloid was determined by drying a sample under vacuum at 60°C. to constant weight.

Freezing Points of Alcohol and Sucrose Solutions and their Mixtures

In Table I are given the freezing point depression data of solutions of sucrose and of ethyl alcohol in water. When these values are plotted against the molalities (a molal solution is taken to signify a solution containing 1 gram mole of solute per 1000 grams of water) it is seen that for alcohol the curve is very nearly a straight line but that the curve for sucrose deviates progressively from a straight line in a manner indicative of a greater apparent degree of hydration of the sucrose molecules at higher concentration, i.e. the sucrose does not attract the H_2O molecule to an extent always equivalent to the formation of a hexahydrate, as has been suggested,⁷ but increases in its apparent degree of hydration with decrease in the relative activity of the water in the solution.

TABLE I

Freezing points of sucrose and of ethyl alcohol of various molalities

Molality	0.1	0.2	0.4	0.5	0.8
Δ , °C.	.189	.380	.765	.975	1.613
Δ , °C.	—	—	—	.910	1.455
Molality	1.0	1.2	1.5	2.0	3.0
Δ , °C.	2.050	2.495	3.182	4.336	6.775
Δ , °C.	1.822	2.183	2.740	3.652	5.420

Abegg⁸ showed that the osmotic work performed by a mixtures of solutes dissolved in a given amount of solvent was generally greater than the sum of the osmotic work done by the same amounts of each solute dissolved separately in the same amount of solvent. The freezing point depression for a mixture of solutes would therefore usually be greater than the sum of the freezing point depressions of the same amounts of solutes dissolved separately in an equal amount of solvent. This is probably to be explained by the fact that most solutes when dissolved show a more or less marked solvation effect, and as the solutions become more concentrated (whether by the same or a different solute) the degree of solvation increases. In Table II are given the freezing point data obtained from mixtures of solutions of sucrose and alcohol, together

⁷ Scatchard: J. Am. Chem. Soc., **43**, 2406 (1921).

⁸ Z. physik. Chem., **15**, 209 (1894).

with the amount of water associated with each solute as calculated in the manner used for finding the amount of water associated with colloids as given above.

In this table, V_a and V_s denote the number of grams of water per 1000 grams total water associated with the alcohol and with the sucrose, respectively, as calculated by the equation given above for calculating the fraction associated with the colloids.

In the calculation of V_a , Δ_a/Δ_s is used in place of $(\Delta_o - \Delta_s)/\Delta_o$ because the excess in the value Δ_o over $\Delta_a + \Delta_s$ is due entirely to the sucrose fraction of the mixture. In calculating V_s , $(\Delta_o - \Delta_a)/\Delta_o$ is actually equal to Δ_s/Δ_o . (Δ_a and Δ_s for these calculations are taken from Table I.) These relationships follow from the differences in form of the freezing point-concentration curves of sucrose and alcohol. From these values of V_a and V_s and the number of mols of the corresponding solute known to be present in the mixture, can be calculated the molality of the alcohol and sucrose solutions, M_a and M_s , which would contain these volumes of water. It is then possible to read, by extrapolation of data in Table I, the freezing points of solutions of the crystalloids of these molalities. This value of the freezing point should coincide with that of Δ_o , the observed freezing point of the mixture. The last two columns give values denoting the accuracy with which this extrapolated freezing point coincides with Δ_o . It is to be pointed out that the results of such calculations are almost 100%, the expected value when the calculation is done with the alcohol fraction of the data, but may vary appreciably from the expected value when the sucrose data are used in the calculation. This is due to the difference in the concentration-freezing point depression curves of these two crystalloids. As mentioned before the curve for alcohol is a straight line but that for sucrose is a curve deviating regularly from a straight line. The calculation from sucrose data would therefore require a more complicated equation than that used for the alcohol data in order to acquire the same accuracy. As will be seen later the results upon the gum arabic are more in accord with expected values when an alcohol solution is used as dehydrating agent than when a sucrose solution is used. The reason for this lies in the above explanation.

For solutions of mixtures of crystalloids, however, the amount of solvent which will be found in association with the mixture, at any given activity of the solvent, is the summation of the amounts which would be found associated with equal weights of the individual crystalloids if they alone were dissolved in the solvent at the same solvent activity. It may be assumed that the same will be true when one of the non-solvent components is a colloid. This assumption is substantiated by the following experiments on such mixtures.

"Bound" Water in Colloid Systems from Freezing Point Data

In Table III are shown the results of an experiment designed to find out if the amount of water associated with unit weight of colloid varied radically with the concentration of the colloid (crystalloid content being constant), or if it varied only slightly, as would be expected from the fact that the activity

of the water in the solutions varies very little with the concentration of the colloid. In Table III the total variation in activity of water between solutions containing one to twenty-five grams of gum arabic per one hundred grams of water is shown to be from 0.98022 to .97680 (corresponding to freezing points of $-2.061^{\circ}\text{C}.$ and $-2.422^{\circ}\text{C}.$, respectively). The last column of Table III shows the grams of water in the solutions which are associated with one gram of bone dry gum arabic. These values indicate that the change in water bound by unit weight of the gum arabic, throughout this short range of activity change in the water of the solutions, is very small, possibly within the range of error of the experiment. There is, however, a noticeable tendency toward a lower binding capacity at the lower activities of the water.

Next it was desired to find if the water "bound" by the colloid could be changed by changing the activity of the solution of crystalloid with which it was in equilibrium. To do this it was only necessary to vary the concentration of the crystalloid while allowing the concentration of gum arabic to remain constant. Tables IV and V show the results of this experiment, first

TABLE III

Freezing point data with solutions molar in sucrose and containing varying amounts of air dry gum arabic. (Bone dry weight of arabic = 84.3% of air dry weight)

Grams Arabic per 100 Gm. H_2O	Grams Sucrose per 100 Gm. H_2O	Δ_o $^{\circ}\text{C}$	$\Delta_o - \Delta_s$ $^{\circ}\text{C}.$	$\frac{\Delta_o - \Delta_s}{\text{Gm. Arabic}}$	$\frac{(\Delta_o - \Delta_s) \cdot 100}{\Delta_o \text{ Gm. Arabic}} = S$	$S \frac{S}{.843} + .1$
0	34.2	2.045	0	0	0	0
1	34.2	2.061	0.016	.0160	.777	1.079
3	34.2	2.091	0.046	.0153	.734	1.039
5	34.2	2.138	0.093	.0186	.869	1.188
7	34.2	2.160	0.115	.0164	.760	1.058
10	34.2	2.192	0.147	.0147	.671	.963
15	34.2	2.288	0.243	.0162	.708	.997
20	34.2	2.368	0.323	.0162	.683	.968
25	34.2	2.422	0.377	.0151	.623	.896

using sucrose and then using ethyl alcohol as the crystalloid. Table VI gives similar data obtained from solutions containing a constant quantity of Na-caseinate and varying concentrations of ethyl alcohol. In column seven of these tables is shown the total grams of water associated with one gram of bone dry colloid in the solutions. Column eight gives the relative activity of the water present in the solutions as calculated from their freezing point depressions.

The gum arabic used in all these experiments was of the same stock sample. The difference in the water content of the samples of gum arabic used in the sucrose and alcohol experiment was due to the fact that the one used in sucrose experiments was the freshly ground gum while that used in the alcohol experiments had been exposed to the atmosphere for several days after grinding and had taken up some water therefrom. In Table VII and VIII respectively,

TABLE IV

Freezing point data with solutions of varying molality of sucrose and constant gum arabic (air dry) content. (Bone dry arabic = 84.3% air dry weight)

Molality of Sucrose	Grams Arabic (air dry) per 100 grams H ₂ O	Δ _o °C.	Δ _a °C.	Δ _o - Δ _a °C.	$\frac{(\Delta_o - \Delta_a) \cdot 100}{\Delta_o \cdot 20} = S$ grams	$\frac{S}{0.843} + 0.157$ grams	Activi of Water a
0.6	20	1.388	1.190	0.198	.714	1.004	.9866
1.0	20	2.368	2.050	0.318	.672	.954	.9773
1.2	20	2.788	2.500	0.288	.517	.771	.9733
1.8	20	4.213	3.880	0.333	.395	.625	.9577
2.4	20	5.718	5.315	0.403	.352	.575	.9459
3.0	20	7.240	6.775	0.465	.321	.538	.9322
0	20	0.090	—	—	—	—	—

are given the vapor pressure data on samples of gum arabic and Na-caseinate taken from the same stock samples as those used in obtaining the data in the other tables shown. In these tables the activity of the water present in the colloid samples is taken as equal to the relative vapor pressure exhibited by that water as determined in the isotenoscope.

Fig. 1 shows these values of the activity of the water associated with the gum arabic plotted against the calculated or observed values (from above experiments) of the weight of water associated with one gram of bone dry colloid. It is to be noted that the values as determined from vapor pressure observations form a continuous curve with those calculated from the freezing point data in sucrose

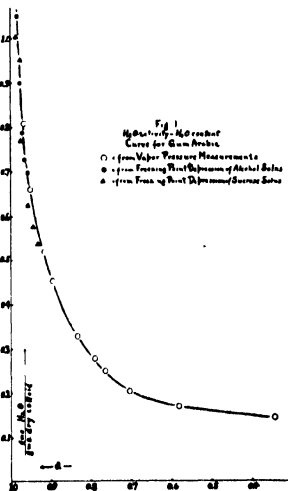


TABLE V

Freezing point data with solutions of varying molality of ethyl alcohol and constant gum arabic (air dry) content. (Bone dry arabic = 87.4% air dry weight)

Molality of Alcohol	Grams Arabic (air dry) per 100 grams H ₂ O	Δ _o °C.	Δ _a °C.	Δ _o - Δ _a °C.	$\frac{(\Delta_o - \Delta_a) \cdot 100}{\Delta_o \cdot 20} = S$ grams	$\frac{S}{0.874} + 0.126$ grams	Activi of Water a
0.38	20	0.894	0.691	0.203	1.141	1.428	.9914
0.76	20	1.657	1.388	0.269	.813	1.054	.9841
1.14	20	2.403	2.078	0.325	.677	.898	.9769
1.52	20	3.140	2.775	0.365	.582	.789	.9700
1.90	20	3.876	3.465	0.411	.530	.729	.9631
2.28	20	4.616	4.154	0.462	.501	.697	.9562
0	20	0.095	—	—	—	—	—

of the water in the solutions varies very little with the concentration of the colloid. In Table III the total variation in activity of water between solutions containing one to twenty-five grams of gum arabic per one hundred grams of water is shown to be from 0.98022 to .97680 (corresponding to freezing points of $-2.061^{\circ}\text{C}.$ and $-2.422^{\circ}\text{C}.$, respectively). The last column of Table III shows the grams of water in the solutions which are associated with one gram of bone dry gum arabic. These values indicate that the change in water bound by unit weight of the gum arabic, throughout this short range of activity change in the water of the solutions, is very small, possibly within the range of error of the experiment. There is, however, a noticeable tendency toward a lower binding capacity at the lower activities of the water.

Next it was desired to find if the water "bound" by the colloid could be changed by changing the activity of the solution of crystalloid with which it was in equilibrium. To do this it was only necessary to vary the concentration of the crystalloid while allowing the concentration of gum arabic to remain constant. Tables IV and V show the results of this experiment, first

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Grams Arabic per 100 Gm. H_2O	Grams Sucrose per 100 Gm. H_2O	Δ_o $^{\circ}\text{C}$	$\Delta_o - \Delta_s$ $^{\circ}\text{C}$	$\frac{\Delta_o - \Delta_s}{\text{Gm. Arabic}}$	$\frac{(\Delta_o - \Delta_s) \cdot 100}{\Delta_o \text{ Gm. Arabic}} = S$	$\frac{S}{.843} + .157$
0	34.2	2.045	0	0	0	0
1	34.2	2.061	0.016	.0160	.777	1.079
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5	34.2	2.138	0.093	.0186	.869	1.188
7	34.2	2.160	0.115	.0164	.760	1.058
10	34.2	2.192	0.147	.0147	.671	.963
15	34.2	2.288	0.243	.0162	.708	.997
20	34.2	2.368	0.323	.0162	.683	.968
25	34.2	2.422	0.377	.0151	.623	.896

using sucrose and then using ethyl alcohol as the crystalloid. Table VI gives similar data obtained from solutions containing a constant quantity of Na-caseinate and varying concentrations of ethyl alcohol. In column seven of these tables is shown the total grams of water associated with one gram of bone dry colloid in the solutions. Column eight gives the relative activity of the water present in the solutions as calculated from their freezing point depressions.

The gum arabic used in all these experiments was of the same stock sample. The difference in the water content of the samples of gum arabic used in the sucrose and alcohol experiment was due to the fact that the one used in sucrose experiments was the freshly ground gum while that used in the alcohol experiments had been exposed to the atmosphere for several days after grinding and had taken up some water therefrom. In Table VII and VIII respectively,

TABLE IV

Freezing point data with solutions of varying molality of sucrose and constant gum arabic (air dry) content. (Bone dry arabic = 84.3% air dry weight)

Molality of Sucrose	Grams Arabic (air dry) per 100 grams H ₂ O	Δ_o °C.	Δ_s °C.	$\Delta_o - \Delta_s$ °C.	$\frac{(\Delta_o - \Delta_s) \cdot 100}{\Delta_o \cdot 20} = S$ grams	$\frac{S}{0.843} + 0.157$ grams	Activity of Water <i>a</i>
0.6	20	1.388	1.190	0.198	.714	1.004	.9866
1.0	20	2.368	2.050	0.318	.672	.954	.9773
1.2	20	2.788	2.500	0.288	.517	.771	.9733
1.8	20	4.213	3.880	0.333	.395	.625	.9577
2.4	20	5.718	5.315	0.403	.352	.575	.9459
3.0	20	7.240	6.775	0.465	.321	.538	.9322
0	20	0.090	—	—	—	—	—

are given the vapor pressure data on samples of gum arabic and Na-caseinate taken from the same stock samples as those used in obtaining the data in the other tables shown. In these tables the activity of the water present in the colloid samples is taken as equal to the relative vapor pressure exhibited by that water as determined in the isotenscope.

Fig. 1 shows these values of the activity of the water associated with the gum arabic plotted against the calculated or observed values (from above experiments) of the weight of water associated with one gram of bone dry colloid. It is to be noted that the values as determined from vapor pressure observations form a continuous curve with those calculated from the freezing point data in sucrose

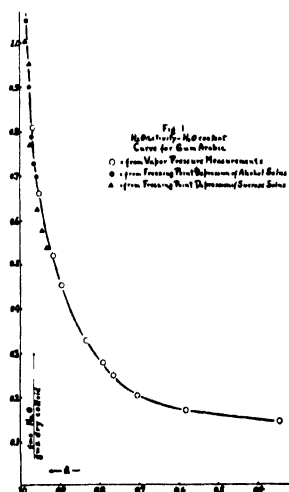


TABLE V

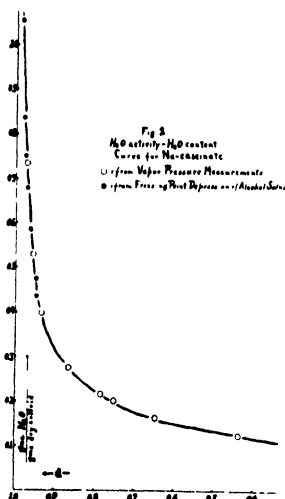
Freezing point data with solutions of varying molality of ethyl alcohol and constant gum arabic (air dry) content. (Bone dry arabic = 87.4% air dry weight)

Molality of Alcohol	Grams Arabic (air dry) per 100 grams H ₂ O	Δ_o °C.	Δ_s °C.	$\Delta_o - \Delta_s$ °C.	$\frac{(\Delta_o - \Delta_s) \cdot 100}{\Delta_o \cdot 20} = S$ grams	$\frac{S}{0.874} + 0.126$ grams	Activity of Water <i>a</i>
0.38	20	0.894	0.691	0.203	1.141	1.428	.9914
0.76	20	1.657	1.388	0.269	.813	1.054	.9841
1.14	20	2.403	2.078	0.325	.677	.898	.9769
1.52	20	3.140	2.775	0.365	.582	.789	.9700
1.90	20	3.876	3.465	0.411	.530	.729	.9631
2.28	20	4.616	4.154	0.462	.501	.697	.9562
0	20	0.095	—	—	—	—	—

TABLE VI

Freezing point data with solutions of varying molality of ethyl alcohol and constant content of Na-caseinate of pH 6.9 (containing 55.5×10^{-5} equivalent of Na per gram of casein). (Bone dry caseinate = 92.3% air dry weight)

Molality of Alcohol	Grams Caseinate (air dry) per 100 grams H ₂ O	Δ_o °C.	Δ_s °C.	$\Delta_o - \Delta_s$ °C.	$\frac{(\Delta_o - \Delta_s) \cdot 100}{\Delta_{0.20}} = S$	$S \frac{8}{0.923} + 0.077$	Activity of H ₂ O
.643	20	1.455	1.192	.263	.903	1.056	.9860
.991	20	2.148	1.847	.301	.701	.836	.9794
1.185	20	2.496	2.186	.310	.621	.751	.9761
1.518	20	3.177	2.824	.353	.555	.679	.9701
1.922	20	3.946	3.576	.370	.508	.585	.9624
2.696	20	5.481	5.078	.403	.367	.475	.9481
2.963	20	5.980	5.583	.397	.332	.437	.9434



and alcohol solutions. The values obtained in the alcohol solutions seem to be somewhat closer to those determined by vapor pressure measurements than are those obtained in the sucrose solutions. However, all agree very closely, considering the differences in the manner in which the results were obtained. Fig. 2 shows the caseinate data in a similar manner. Here, too, the agreement is very good, verifying the applicability of the theory to quite a different type of colloid.

Theoretically any crystalloid could be used as readily as the two which have been used in these experiments so long as no reactions occurred between it and the colloid. Table IX, however, shows that not all crystalloids may be so used.

Of the eight crystalloids used in this experiment it is found that the values gained with methyl alcohol, acetic acid and glucose compare well with the values obtained when sucrose and ethyl alcohol were used. Gum arabic is the colloid used in these determinations. However, chloral hydrate and acetamide give values somewhat low and sodium and potassium chlorides give values which are very low. It is probable that the electrolytes cause a depressing of the degree of ionization of the gum arabic and that this accounts for the abnormally low value calculated from the freezing point data with these substances. In the case of the non-electrolytes which give low values, it is probable that some sort of physical compound is formed between the crystalloid and colloid which serves to lower the activity of either or both in their effect upon the activity of the water in which they are dissolved. The value ob-

TABLE IX
Freezing point data with solutions of various crystalloids having 20 grams gum arabic content per 100 grams H₂O. (Bone dry weight of arabic = 87.3% air dry weight)

Crystalloid	Molality Crystalloid	Δ_e °C.	Δ_o °C.	$\Delta_o - \Delta_e$ °C.	$\frac{(\Delta_o - \Delta_e) \cdot 100}{\Delta_o \cdot 20} = S \frac{S}{.873} + .127$ Grams	Activity of Water a	Value extra- polated from curves in Fig. 1 Grams
Methyl alcohol	1.312	2.339	2.573	.234	.605	.9754	.850
Glucose. 1 H ₂ O	1.000	1.934	2.137	.203	.636	.9795	.925
Chloral hydrate	1.000	1.880	2.033	.153	.501	.9805	.950
Acetamide	1.000	1.935	1.962	.127	.432	.9812	1.000
NaCl	1.000	3.317	3.462	.145	.279	.9670	.760
KCl	1.000	3.159	3.267	.108	.220	.9688	.790
HAc	1.000	1.810	2.001	.191	.636	.9808	.950
Urea	1.000	1.786	1.731	.055	—	.9834	—

tained with urea is extraordinarily low. Urea is noted for its peculiar effects in colloid systems. No satisfactory explanation for its action can be offered.

From the results of the above experiments it is quite obvious that the "bound" water cannot be thought of as consisting of a constant fraction of the total water.

So long as only one value for the relative activity of the water with which the colloid exists in equilibrium is considered, this concept might be considered as correct. At any other value for the relative activity of the water at which the "bound" fraction may be determined, however, a different value will be obtained for this "bound" fraction. It is always necessary to define the relative activity of the water in the system before it is possible to speak of a definite fraction of it as being associated with or bound to any one of the non-water components. At some definite activity it can be said that the water is distributed according to a certain ratio between the crystalloids or colloids which make up the non-water fraction of the solution. If the H_2O activity- H_2O content curve is a straight line for each and every one of these components as is approximately the case in mixed solutions of crystalloids, then the same ratio will hold at all activities of the water. But if these curves are not straight lines, as is to a small extent the case with crystalloids and as is most radically the case with colloids, then the ratio of the water associated with these non-water components will vary (in case of colloids, radically) with the activity of the water in the system. However, at any given activity of the water in a

TABLE VII

Vapor pressure data on gum arabic samples of varying water content

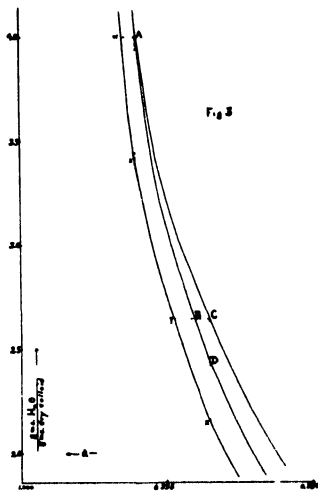
$\frac{\text{Grams } H_2O}{\text{Grams dry colloid}}$	Observed vapor Pressure at 25°C. mm. Hg.	Relative vapor pressure or activity of H_2O a
.663	23.33	.950
.810	22.72	.966
.502	21.56	.916
.454	21.05	.895
.329	19.60	.833
.279	18.57	.789
.250	17.95	.763
.203	16.55	.704
.144	8.04	.343
.169	13.65	.581

system in which colloids and crystalloids are in equilibrium, provided the various non-water components have not reacted with each other upon mixing, the amount of water associated with or bound by a given weight of any of the non-water components will always be the same, and it will be the same as that which the given weight of the component will be associated with at the same water activity when it is the only non-water component in equilibrium with water.

The definition of "bound" water offered by Gortner and Newton and by Hill is not complete until the activity of the water in the system upon which the measurement is made is taken into account; the "free" or "bound" fractions of the total water with reference to any non-aqueous component, such as the colloid component, of the system will be found to vary with the activity of the water present in that system.

Hill found that the amount of water which was, according to his definition, "bound" to the colloids present in blood or muscle tissue was of the order of 3% of the total water normally present in those tissues or about 0.1-0.2 grams of water per gram dry solid. This value is quite low when compared with the values which have been obtained from vapor pressure measurements on isoelectric, ash free proteins (see casein in preceding paper of this series) at activities of water comparable to that present in blood or muscles.

Fig. 3 illustrates, in graphic form, the meaning of Hill's measurements in terms of the water activity-water content curve which has been offered in this paper as the theoretical basis upon which the methods used for the determination of "bound" and "free" water in colloid systems may be explained. The same analysis applies exactly to the method of Gortner and Newton. Where *activity depressions* are used in this graphical analysis, Gortner and Newton used *freezing point depressions* and Hill used *vapor pressure depressions*.



Suppose the curve AC' , in Fig. 3, to represent a segment of the true H_2O activity- H_2O content curve for the mixture of crystalloids and colloids present in blood, or muscle, which consists roughly of nineteen parts colloid, one part crystalloid and eighty parts water. Thus the point A would represent the activity of the water in the original tissue or fluid, at which point the water content was equal to four grams per one gram of solid. If, now, at point A on the curve, we could assume that the solids present were all acting as crystalloids and the system was following the laws of dilute solution, we would find that the water activity-water content curve would follow the curve ABD instead of the curve AC . The activity increment BC indicates the extent to which the curve AC has deviated from the laws of dilute solutions between points A and C in the water content of the system. The water content increment CD is a measure of the amount of water present in the system which appears to be not available to the system if it is assumed to be obeying the laws of dilute solution.

The water content corresponding to point D would indicate the "free" water in Hill's or Gortner and Newton's measurements while the increment

CD would measure the "bound" water. Further consideration of the graph shows, however, that these measures of "free" and "bound" water are in no sense absolute. In the first place, the assumption that the entire activity depression corresponding to point *A* has been brought about according to the laws of dilute solutions is not correct. As a matter of fact, as the water activity-water content curve is followed down from the infinite water content (at which the H_2O activity would equal 1.0) it is found to deviate, from the beginning, from the curve which would be followed were the system obeying strictly the laws of dilute solutions, so that, when a water content of 4 grams per gram of solid is reached, the curve would already have deviated a distance of, say, *wA* from the dilute solution curve *wxyz*. To measure the water associated with or "bound" to the colloid in the system at any given activity it would be necessary to measure the water content increment lying between the curves *AC* and *wxyz* and not between *AC* and some arbitrary curve as *ABD*. Of course, we would have to assume, too, that the entire deviation between curves *AC* and *wxyz* was due to the colloid present. This is not strictly permissible; some crystalloid solutions deviate likewise to a noticeable extent. But the degree of error arising from such an assumption will be small, by far the greater part of such deviation being due to the colloid present. Thus, at an activity corresponding to point *A*, the water "bound" by the colloid fraction would be equal to the increment *Ax* while at activity *C*, it would equal to *Cz*. And this water would truly be that "bound" by the colloid. It would correspond to the amount "bound" by the isoelectric or ion free colloid, because any ions which might be attached to the colloid would follow the laws of dilute solutions in their relationship with the water present and would thus have no part in causing the deviation observed between curves *AC* and *wxyz*.

It is obvious that the "bound" water, or better, the water associated with the colloid, as determined by either Gortner and Newton's or Hill's methods will be lower than that which is actually associated with the colloid. Likewise different values could be gained, at the same activity (at point *C*) of the water present, by conducting the experiment in such a manner that the activity difference between the initial point, *A*, and the final point, *C*, was different from the points arbitrarily chosen by these investigators. (In Gortner and Newton's method the point *A* corresponds to the freezing point of the original plant sap and the point *C* to the freezing point of the original sap plus enough sucrose to form a molar solution. With Hill's method point *A* is arbitrarily taken as equal the relative vapor pressure of Ringer's solution while point *C* corresponds to the relative vapor pressure of approximately a $3/2$ Ringer's solution.) And, as pointed out before, the absolute amount of water associated with the colloid will vary with the activity (i.e. with shift in point *C*) of the water with which it is in equilibrium.

Rubner's Method

The methods used by Gortner and Newton and that of Hill have yielded to a satisfactory explanation in terms of the relative vapor pressure-water content curve of the colloid. But the method described as that devised by

Rubner, and which has been used by Thoenes and Robinson, fails to succumb completely to this explanation.

It is obvious from the above considerations that the activity at which the "bound" water (in this case, the unfrozen water) is being determined by this method is that activity which is equivalent to a freezing point of -20°C . This is equal to an activity of water of 0.8221. When equilibrium has been attained at this freezing temperature the water remaining unfrozen must have an activity of 0.8221. By this method no differentiation is made between the water which is associated with the colloid and the crystalloid fractions of the non-water components of the sample. It is therefore of no value for estimating the amount of water which might be associated with the colloid alone. A NaCl solution which freezes at -20°C . and exhibits a relative vapor pressure of 0.8221 will be found to contain 3.46 grams of water per gram of salt. If any solution of NaCl is subjected to a temperature of -20°C . and the amount of water which does not freeze is determined by the above method, it should be found that for every gram of NaCl in the sample there should remain 3.46 grams of water unfrozen, i.e. "bound" so strongly as to be unremovable under the desiccating force (equivalent to a relative vapor pressure of 0.8221) applied. The same should be true at -15°C . except that for

TABLE VIII

Vapor pressure data on Na-caseinate (pH = 6.9) samples of varying water content

Grams H_2O Grams dry solid	Observed vapor pressure at 25°C . mm. Hg.	Relative vapor pressure or activity of H_2O a
.734	22.90	.974
.530	22.50	.957
.399	22.00	.935
.277	20.35	.865
.216	18.45	.784
.204	17.65	.751
.164	15.20	.647
.124	10.30	.438
.060	4.25	.180

every gram of salt there would be 4.50 grams of water left unfrozen, since -15°C . is equivalent to a relative vapor pressure of 0.8636 and a NaCl solution which has this relative vapor pressure contains 4.50 grams of water per gram of the salt. And so at any other freezing temperature to which the solution might be subjected, the amount of water left unfrozen will be such as to form a solution of salt which shows a relative vapor pressure equivalent to that freezing point depression.

An experiment was performed by this method upon NaCl solutions of various concentrations.¹ The amount of water which should be left unfrozen in the solutions, was calculated from the freezing point-concentration data

¹ The writer is much indebted to Dr. Wm. Robinson for the experimental data reported for this method.

TABLE X

Unfrozen water left in salt solutions of varying concentrations of NaCl when subjected to freezing temperatures -20°C. and -15°C.

Weight of NaCl in 1 Gm. Soln.	H ₂ O in 1 Gm. sample	Grams H ₂ O unfrozen per gram NaCl at -20°C.		Obs./Calc. at -20°C.	Grams H ₂ O unfrozen per gram NaCl at -15°C.		Obs./Calc. at -15°C.
		Calc.	Obs.		Calc.	Obs.	
.005	995	0173	0174	1 006	0225	0260	1.155
009	991	0311	0326	1 048	0405	0443	1.093
.017	983	0588	0551	938	0765	0849	1.110
.025	975	0865	1049	1 213	1125	1310	1.164

for NaCl obtained from Landolt-Börnstein's Tables. Table X shows the results as determined at two freezing temperatures, i.e. -20°C. and -15°C.

The formula used for calculating the frozen "free" water from the calorimetric data is

$$X = \frac{C(T_1 - T) - AS(T_0 + T)}{80 - T_0}$$

where X = the grams of unfrozen water in sample containing *n* grams of non-water components,

C = the heat capacity of the calorimeter,

A = total weight of wet sample,

S = specific heat of the wet sample,

T = final temperature of calorimeter and sample,

T₁ = initial temperature of calorimeter before adding frozen sample,

T₀ = initial temperature of frozen sample (negative sign disregarded)

If *w* = the total water present in sample then *w* minus X = the water left unfrozen or "bound," and *w* - X/*n* = the grams of "bound" water per gram of dry solid.

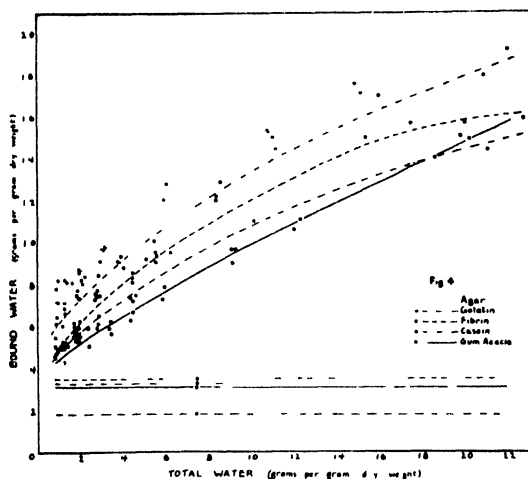
When the fact is considered that these observed results were calculated from calorimetric data obtained in samples (in triplicate) of salt solutions weighing less than a gram, the correlation between the observed and calculated values is very good. This, of course, indicates that the interpretation of "bound" water as measured in this manner is that suggested above, namely it is the amount of water necessary to form a solution with the non-water constituents which will exhibit a relative vapor pressure of 0.8221 or a freezing point depression to -20°C.

When samples of colloids were used in place of NaCl solutions, however, the results could not be so simply explained. From their relative vapor pressure-water content curves, the amounts of water which were found to be associated with 1 gram of the dry colloids, at a relative vapor pressure or water activity of 0.8221, were the following:

Agar (Merck)	0 37 gm.
Fibrin (crude)	0 33 gm.
Gelatin (commercial)	0 33 gm.
Gum Arabic (commercial)	0 32 gm.
Casein (nach Hammarstein)	0 18 gm.

These are, theoretically, the amounts of water which should remain unfrozen per gram of dry colloid when the sample had been allowed to come to equilibrium at the temperature of -20°C . The initial concentration of the colloid in the sample should have no effect upon the amount of water left unfrozen per gram of colloid when this equilibrium has been reached. To what extent the observed values for the water "bound" by the colloids, as determined by this method, differ from the theoretical values given above is shown graphically in Fig. 4, where in the grams of water left unfrozen (or "bound" by one gram of the dry colloid) are plotted against the grams of total water present per gram of dry colloid in the sample.

It seems that the water "bound" per gram of dry colloid increases with dilution and would only approach the theoretical value when the original content of water was equal to the theoretical value, i.e. when no ice would be formed at all. There is little doubt that equilibrium has been attained, since samples left at -20°C . for a week or more show no significant difference from those left only 3 or 4 hours.



When these unexpected higher values for "bound" water were obtained it was first thought that there might be some heat of imbibition of the colloid as the water which had been frozen out was again allowed to thaw in the presence of the colloid. An analysis of the heat of imbibition-water content curves determined by Katz⁹ for several colloids indicated, however, that after the water in the colloid had reached an amount such that it exhibited a relative vapor pressure of 0.8221 (equivalent to a freezing point -20°C .) any added water caused almost no perceptible evolution of heat. Actual experiment on gum acacia substantiated this conclusion.

As yet, no explanation for these unexpected values can be given.* It may be due to a protective action of the colloid whereby equilibrium is indefinitely delayed. It may be that the colloid causes formation of microscopic ice crystals to such an extent that interfacial energy relationships would cause

⁹ Kolloidchem. Beihfte, 9, 1 (1917).

* Note: Data obtained subsequently to the presentation of this paper have failed to substantiate the findings reported for this method and shown graphically in Fig. 4. For gum arabic the curve is found to be a straight line parallel to the horizontal axis but in the region of 0.40 grams H_2O instead of 0.32 grams as required by theory. The method is subject to a large number of errors and it is difficult to make accurate and repeatable determinations, especially when the sample contains a gelatinous material. Since the "bound" water value is always a small difference between total and freezable water, all errors of the method are concentrated therein and the percentage variation is quite large.

a shift in the equilibrium point. The fact that the theory holds when measurements are made on crystalloid systems and does not when applied to colloid systems, would seem less to detract from the accuracy of the theoretical picture than to point to some unexpected and unaccounted for influence that the colloid has had upon the formation of ice crystals.

One other method for the determination of bound water may be mentioned and shown to be interpretable in terms of this general picture. Taylor¹⁰ devised a method for estimating bound water in colloids by placing the colloid in a phenol solution of which the temperature was known at which the two phases, water and phenol, separated upon cooling. The colloid would cause a shift in this temperature by an amount bearing a constant relationship to the amount of water which the colloid "bound" or became associated with. It is the same phenomenon which is observed when a colloid is put into a sucrose solution and found to change the freezing point of that solution. The water in the phenol solution will have an activity less than that of pure water and the colloid will be found to have a definite amount of water associated with or "bound" to it at that activity of the water. If it is found that the phenol has had no effect upon the chemical nature of the colloid, the amount of water which the colloid will hold at this particular activity will be the same as it would hold in a sugar solution in which the water shows the same activity, or, that it would be found to contain when mixed alone with water showing a relative vapor pressure of the same value.

Summary

A theoretical basis for "bound" water determinations in colloid systems is outlined in terms of the relative vapor pressure-water content curve (vapor pressure isotherm) of the colloid.

"Bound" water is defined as that portion of the water in a system containing colloid and crystalloid which is associated with the colloid together with those ions which form a part of the colloid complex. "Bound" water is not a fixed quantity of water associated with the colloid but will vary with the activity of the water in the system in a manner consistent with the vapor pressure isotherm of the colloid.

At any given activity of the water in a system in which colloids and crystalloids are in equilibrium, provided the various non-water components have not reacted with each other, the amount of water associated with or "bound" by a given weight of any of the non-water components will always be the same, and it will be the same as that which the given weight of the component will be associated with at the same water activity when it is the only non-water component present in the system.

Some of the methods which have been used for the determination of "bound" water are interpreted in terms of this picture.

Chicago, Illinois.

¹⁰ J. Physiol., 67, 39 (1929).

FREE AND BOUND WATER IN ELASTIC AND NON-ELASTIC GELS*

BY IVAN D. JONES AND ROSS AIKEN GORTNER

Introduction

The more recent trend in all studies of colloidal systems has been toward an application of physico-chemical methods, in order that the reactions which take place under given conditions may be defined more exactly and be determined quantitatively. Later investigations in the narrow field of studies on the binding of water by substances in the colloidal state have been in keeping with this modern approach to problems in colloid chemistry.

The ability of certain substances in the colloidal state, when in the presence of a given liquid, to hold a quantity of that liquid with great forces of attraction has been long recognized. The exact nature and intensity of these attractive forces have, to date, not been clearly defined.

This attraction, when exhibited toward water, results actually in a hydration of the colloidal particles and gives rise to the phenomenon known as water-binding. Water-binding or hydration is accompanied with certain physical and chemical changes in the colloidal system. Thus, the viscosity is relatively greatly increased, the vapor pressure may be markedly reduced, and a certain portion of the water in the system will no longer act as a solvent.

Foote and Saxton^{13,14,15} concluded that a definite portion of water would not freeze from the inorganic hydrogels which they studied and the investigations of Rubner⁵² and Robinson^{48,49,50} were based on the hypothesis that at temperatures as low as -20°C bound water would not freeze.

It is the purpose of the present paper to report a study of the relation of temperature to the quantities of water which appear to exist in the "free" condition, and in the "bound" or "unfree" condition at known temperatures below the freezing point of a given colloidal system. Also, the behavior upon repeated freezings at low temperatures of certain organic hydrosols and hydrogels is compared with that of certain inorganic colloidal systems.

Historical

Since the early investigations of colloidal systems were generally of a qualitative nature and only in the more recent studies has quantitative technic been introduced, in reviewing the literature dealing with the effects produced on colloidal systems by freezing, we will consider first qualitative and later quantitative observations.

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Qualitative studies on the effect of freezing on colloidal systems.—Records are available of observations on the effect of freezing of colloidal substances which antedate the recognition of the phenomena of the colloidal state by Thomas Graham. Thus in 1820 Vogel⁶³ noted that a distinct change in wheat starch paste occurred upon freezing. When the frozen paste was thawed, a liquid containing but little dissolved substance separated from a spongy mass.

Molisch³⁶ in 1897 reported a microscopical study of the freezing of a 2% gelatin sol. He found that on freezing, the gelatin formed a net-work, the meshes of the net being filled with ice-masses and air bubbles. Upon thawing the frozen mass, he observed that the gelatin possessed a sponge-like appearance.

Similar studies, upon gum tragacanth, gum arabic, egg albumin, and tannins were made by Molisch.

Bobertag, Feist and Fischer,² and Fischer and Bobertag¹² considered that freezing produced, in hydrosols of gelatin, agar-agar, soap, and carrageen moss changes which were only partially or very slowly reversible upon thawing.

Callow,⁷ in a study of the rate of ice crystallization through super-cooled gelatin gels, maintained that the "separation of ice irreversibly ruptures the structure of the gel." This conclusion was drawn from observing the behavior of the gels upon repeated freezings.

Inorganic hydrosols have been studied by many workers. The results obtained have not been in complete agreement. Bobertag, Feist and Fischer² observed that upon the thawing of ferric hydroxide sols, frozen at -10°C and -70°C , the sol was re-formed. Lottermoser³² observed that this sol, dialyzed until it had a specific conductivity near that of water, was completely precipitated by freezing. He concluded that the electrolyte present in an undialyzed sol served as a stabilizing agent. By the freezing of incompletely dialyzed ferric hydroxide sols, he was able to effect only a partial precipitation of the colloidal material.

Lottermoser and also Bruni⁶ independently observed the effect of freezing upon the hydrosol of silicic acid, and reported that upon thawing the frozen sol it separated into a clear liquid and a precipitate.

Vanzetti⁶² from repeated experiments with silicic acid gel frozen at temperatures as low as -200°C concluded that the final composition of the gel was independent of the temperature of freezing. A certain portion of the water present could not be removed by freezing.

Liesegang³⁰ repeated Molisch's experiment with a 2% gelatin sol and reported very different results. He found that the water on freezing formed fernlike ice patterns. On thawing, the patterns remained in finest details, and he adds that the most gelatin was found where the most ice had appeared. Apparently, therefore, water was not separated from the gelatin upon freezing and subsequent thawing. Stiles⁶⁶ has raised the question as to whether the differences in results obtained by Molisch and by Liesegang were not caused by different rates of freezing of the gelatin sol.

In an excellent treatment of the fundamental physical and chemical principles of the freezing process Stiles⁵⁶ has definitely shown that the conditions under which freezing takes place determine, to a very large degree, the effects which will be produced by freezing. His work was based on the early investigations of Tammann⁶⁹ who concluded that in the crystallization of a super-cooled liquid, two factors were important, (A) velocity of crystallization and (B) the number of centers from which crystallization takes place.

Moran³⁷ in microscopical studies of the effect of freezing on gelatin gels, found that the structures produced in the gel depended upon the rate of freezing and the concentration of the gel. It is of particular interest that Moran demonstrated that centers of crystallization could be either external or internal, this condition being determined by the temperature at which freezing began. Hardy²¹ carried on further microscopical investigations with frozen gelatin gels, and states "so far as my observations go, when crystals (internal) of pure ice melt, the water is reabsorbed at once by the surrounding gel, leaving only a tiny cleft."

These studies of Stiles, Moran, and Hardy offer an explanation of Liesegang's observations which were at variance with the earlier studies of Molisch.

Hardy^{19,20} advanced the theory that with gels or sols, dehydration could be considered a reversible process, if a gel or sol resulted normally from the addition of a colloidal substance to water; and that it would be an irreversible process, if special conditions were required to produce the gel or sol. Fischer¹¹ in 1911 pointed out that the freezing process might similarly be considered as either reversible or irreversible, since freezing is only a special type of dehydration.

Quantitative Studies on the Effect of Freezing on Colloidal Systems.—Müller-Thurgau³⁸ in 1880 published the results of investigations on apple and potato tissue. In his studies the material was first frozen in an ice and salt bath and then introduced into a water calorimeter. From the quantity of heat required to melt the ice in the sample, he was able to calculate the quantity of water which had been frozen. As has been noted by A. Kuhn,⁹⁹ Müller-Thurgau's first results were undoubtedly erroneous, for he gave no consideration to the molecularly dissolved substances in his samples. The principle involved in Müller-Thurgau's studies has, however, been of great value in that it laid the foundation for much biological research.

Foote and Saxton^{13,14,15} introduced dilatometric technic into studies of the freezing of colloids. They chose as systems for investigations, the inorganic gels of silica, alumina, colloidal ferric hydroxide, and a mixture of lampblack and water. With every system studied they found that a certain portion of the water present remained unfrozen. They accordingly concluded that water in the inorganic hydrogels existed in three forms: (A). Free water—the water which froze between the temperatures 0°C and -6°C. (B). Capillary water—that portion of the water in the sample which froze at temperatures below -6°C. (C). Combined water—the portion of the water which represented the difference between the total water in the sample and the water which could be frozen.

Applications of the dilatometer method have been made by several investigators, notably by Bouyoucos^{3,4} in soil studies, McCool and Millar²³ in soil and plant relationships, and by Rosa,⁵¹ and Lott³¹ in winter-hardiness studies.

Parker,⁴⁶ in 1921, reported some exceedingly important observations on the effect of finely divided insoluble material on the freezing point of different liquids. He found that with water the magnitude of the freezing point depression depends upon at least two factors, (1) the relative surface area of the solid, or the degree of subdivision of the material and (2) the affinity which exists between solid material and water.

Newton and Gortner⁴³ in 1922 suggested a new method for the quantitative measurement of water held in the "unfree" or bound condition in sols of hydrophilic colloids. This method is based on the hypothesis that a certain portion of the water in a hydrophilic sol is associated with the colloid in such a form that it will no longer act as a solvent, as contrasted with "free" water. The addition, then, of a definite quantity of a soluble material to a sample containing a known amount of water would cause a lowering of the freezing point of the mixture, in proportion to the molar concentration which resulted, and a positive deviation of the observed from the theoretical freezing point depression would be a measure of the bound-water which was present. Kruyt and Winkler²⁸ have verified the findings of Newton and Gortner regarding the effect of the presence of hydrophilic colloids on the freezing point depression of solutions of molecularly dissolved substances.

The Newton-Gortner method has served as a quantitative measure of bound-water in many plant investigational projects and related studies, *e.g.* Newton,^{39,40,41} Harris et al.,²² Newton and Cook,⁴² Newton and Martin,⁴⁴ Martin,³⁶ and Crist.⁸

Kuhn²⁹ cites an extensive study made by Rubner of water-binding in *I ammaria* and materials of animal origin. Rubner⁵² employed a method very similar to that first used by Müller-Thurgau, and considered the latent heat of fusion of ice to be a measure of the quantity of water which could be frozen. Thoenes⁶⁰ studies on the effect of freezing upon water-binding in gels and animal tissues involved an experimental procedure modeled closely after that of Rubner.

Thoenes concluded that the method yielded reproducible values when dealing with animal tissues but that these values were only relative. It gave a measure of water-binding intensity but not a true measure of the quantity of water which existed in the bound form in the living organism.

Robinson,⁴⁸ in a study of winter-hardiness in insects, employed the method described by Thoenes. He improved the technic⁴⁹ and pointed out that this method yields only minimal values for bound-water. He demonstrated in a striking manner the importance of bound-water studies in animal physiology.

Moran³⁷ made quantitative investigations on the effect produced by freezing gelatin gels at different temperatures. Having noted that ice formation was entirely external under certain conditions of freezing, Moran

took advantage of the fact that the ice appearing in such manner could be removed from the resulting partially dehydrated gel sample, and thereby he determined the phase equilibrium between ice and gel at different temperatures. He found the equilibrium completely reversible in the gel concentrations which he studied.

Hill,²³ and Hill and Kupalov²⁴ have recently studied the bound-water problem in animal tissues using the vapor pressure method. They arrive at results which are not in agreement with those reported by Thoenes,⁶⁰ who found relatively large amounts of bound-water to be associated with the hydrophilic colloids of muscle, whereas Hill finds very little if any bound water in either blood or muscle. It seems probable that this finding may in part be explained by the technic used and by the assumptions which Hill made. Since Briggs⁵ is presenting on this same program an extensive study of the vapor-pressure method, the reader is referred to his paper for a discussion as to the possible causes of the divergent conclusions noted above. That the majority of physiologists, physicists and physical chemists agree that some sort of water binding occurs in hydrophilic colloid systems is indicated by the extended discussion which was provoked following the recent presentation before the Faraday Society of a summarizing paper on "bound" water by Gortner.¹⁸

Experimental

The Problem.—It is apparent from the preceding historical review that there is not complete agreement as to the effects which are produced when colloidal systems are frozen.

Similar lack of agreement is found when dealing with methods of measurement of bound water. Freezing of colloidal systems has been employed by some investigators as a method in studying the water-binding in certain materials. Foote and Saxton,^{13,14,15} Rubner,⁵² and Robinson^{48,49,60} considered the freezing method as a quantitative measure of water-binding capacity in the materials under investigation. Thoenes⁶⁰ concluded from a study made by means of the Rubner method that it did not give absolute values for water-binding capacity. Kuhn²⁹ has emphasized, in a very complete review of water binding in colloids, that freezing reduces water-binding in colloidal systems, also, that any freezing method is reliable only to the extent that it measures the intensity with which water is bound, and, accordingly, can not be considered a quantitative measure of water-binding capacity.

Gortner¹⁷ (p. 227 *et seq*), in a general discussion of the problem, suggests that the bound water is probably present in the form of oriented dipoles and adds¹⁸ that, "I do not believe there is any sharp line of demarcation between "free" water and "bound" water, but that we must postulate an insensible gradation between molecules of water having the normal activity of pure water and molecules of water where this activity has been so reduced that such molecules have become to all intents and purposes a part of the solid upon which they are adsorbed. One method of measurement may be sensitive enough to differentiate between water molecules having a given activity and those molecules having a greater activity. Another method of measurement

may give somewhat different results because it draws the dividing line at different activity values. What we in biochemistry need at the present time are extensive series of measurements carried out on many biocolloid systems by many different techniques. When these data have all been accumulated, then perhaps it will be time enough to attempt to theorize as to the exact nature of "bound" water."

The purpose of the present paper was to determine with certainty whether or not "bound-water" would freeze, and if so, at what temperature this freezing would take place. If bound-water would not freeze, this process could be employed as a quantitative measure of the bound-water content of hydrophilic colloidal systems. If bound-water would freeze, it was considered that a relationship should exist between the rupture of the gel structure and the freezing of bound-water.

Newton and Martin,⁴⁴ employing the Newton-Gortner method of bound-water measurement with different concentrations of organic hydrosols, found that with increasing concentration of the colloid, there was an increase in the quantity of water which was "bound." This is taken as evidence to support the supposition that gel formation results when the bound-water represents an appreciable portion of the total water present. If then, the bound-water content can be reduced by freezing, a partial or complete rupture of the characteristic gel structure exhibited by certain colloidal systems should result.

The Method.—In order to observe the behavior of hydrophilic colloidal systems upon freezing, it was desirable to employ a method which would permit continuous readings to be taken on a given sample at any desired temperature. Such a method should permit a study of the behavior of the system as the temperature was lowered below its freezing point and also as the temperature was raised again to the melting point of the ice. The dilatometer method was accordingly chosen.

The principle of the dilatometer method is well known and may be summarized as follows:

In any given system changes in temperature are accompanied by corresponding density changes of the system. If with a temperature change, there occurs a liquid \rightleftharpoons solid transition, this is, in general, accompanied by a corresponding change in density. If the mass of the system be fixed, the density changes may be followed by the volume changes which occur.

With water, solidification takes place at 0°C with a density change²⁵ from 0.9999 to 0.9168. This density change results in a volume increase of 0.09074 cc. per gram of water.

Therefore, by measuring the expansion produced by freezing, it is possible to calculate the quantity of water which was frozen. If freezing occurs gradually as the temperature is lowered, there will occur simultaneously (A) an expansion resulting from the gradual formation of ice and (B) a contraction resulting from the temperature lowering of the entire system. Knowing the density change for all solids and liquids in the system for any given temperature change, the ice formation which results from a given temperature change can be calculated.

As has been indicated, other workers have used dilatometric technic in studying ice formation in colloidal systems. They have, in general, calculated the amount of water in the system from a moisture determination of the sample under investigation. In addition the quantity of water which was frozen at a given temperature has, in general, been calculated directly from the expansion which resulted from the freezing process.

In the greater part of this investigation, a known quantity of pure water was pipetted into the dilatometer, and the volume change produced by cooling the system to definitely recorded low temperatures was measured. Following the determination of such "water curves" the same quantity of water was added to small amounts of the solid material under investigation in the dilatometer, and the expansion and contraction resulting from exposures to low temperatures were again measured. Thus, in the second series of measurements the conditions were identical with those prevailing in the first, the only difference in the system being the small quantity of material under observation. It was therefore assumed that the two resulting curves were directly comparable, and if the material did not have the ability to "bind" water, that the second curve would coincide exactly with the first one. Further, it was considered that the measure of the quantity of the water which the material would bind would be indicated by the extent to which the two curves deviated from each other. It was expected that gradually lowering the temperature would cause a convergence of the curve resulting from the freezing of the colloidal system toward the curve for pure water. If such convergence did not occur, then one could rightly assume that decreasing temperatures did not alter the amount of bound water. Stated differently, if at the point of initial freezing all water which would freeze was transformed into ice, then the curves resulting from plotting the readings obtained at different temperatures below that of freezing would be a line parallel to that given by the pure water sample. Any deviation from such a parallel line would be expected to take place in the direction of the pure water line, as it would thereby indicate a decrease in the total quantity of water held in the non-frozen state.

Description and calibration of the apparatus.—The apparatus consisted of a bulb-capillary-stem dilatometer mounted on an engraved meter stick as shown in Fig. 1, and a low temperature thermostat in which the desired low temperature could be produced and maintained.

The dilatometer figured was the most satisfactory and most sensitive one of many designed for these studies. The bulb of the instrument was blown from pyrex tubing about 0.5×3 cm. One end was sealed shut; the other end was fitted with a carefully ground glass stopper which was held firmly in place by means of rubber bands. The stem about 55 cm. long was of pyrex capillary tubing having a 1 mm. bore and was attached to the center of the bulb at a direction perpendicular to its long dimension.



FIG. 1
The dilatometer
as used in this
study

The dilatometer had a capacity of about 5 cc. The sample taken usually occupied a volume of from 1 to 3 cc. The remainder of volume of the dilatometer bulb and a portion of the stem were filled with pure toluene.

Toluene was chosen because of its known coefficient of thermal expansion,²⁵ its high boiling point, and its inactivity toward aqueous colloidal systems. The high coefficient of thermal expansion of toluene produced a decided density change with a small temperature change, therefore serving to counterbalance the expansion produced by the freezing of the water in the sample and made possible the use of a relatively short stem for the dilatometer.

The stem of the dilatometer was attached perpendicularly to the bulb primarily to avoid a possible fracture of the bulb resulting from the expansion of the sample when it froze, and also to facilitate the filling the dilatometer, mixing the sample, and freeing the system from air bubbles.

The volume of the dilatometer was calibrated at room temperature by means of weighing the mercury it would contain. The diameter of the capillary stem and the uniformity of its bore were determined by measuring with a microscope equipped with a movable stage the length of a weighed mercury thread in consecutive sections of the tube. All variations in the bore of the selected capillary were found to be well within the limits of experimental error. The actual diameter of the uniform capillary was further checked by weighing a thread of mercury which filled the greater portion of the length of the tube. The capillary chosen had a volume of 0.0096078 cc. per cm. length.

It was possible to read the length of the toluene column in the capillary with an accuracy of 0.05 cm., thus permitting an accurate measurement of a volume change as small as 0.00048 cc.

The low temperature thermostat consisted of a well-insulated double-walled container, the inner vessel having a capacity of approximately 1 liter. The cooling medium was alcohol, the temperature of which was lowered as desired, by adding a sufficient amount of solid carbon dioxide, similar to the technic suggested by Dunn.⁹ In order to assure uniformity of temperature in the bath the alcohol was stirred continuously by a small electric motor. It was essential to maintain a uniform temperature in the bath, since the temperature of the bath was taken to be the temperature of the system inside the dilatometer after the contents of the dilatometer had arrived at a temperature equilibrium. The size and construction of the dilatometer prohibited the use of a thermometer inside of this instrument.

A 0°C to -65°C pentane thermometer was used in determining the temperatures. This thermometer was checked against a thermometer which had been standardized from a Bureau of Standards certified instrument.

Materials. The materials employed in this investigation fall into two classes. (A) those substances which form gels of the elastic type and (B) those which form inelastic gels.

Gelatin was chosen as a typical substance which forms an elastic gel. Gelatin was especially suitable from many standpoints, two of the factors being its behavior in cold water and its ready sol \rightleftharpoons gel transformation.

Since the method of mixing water with the dry sample was that of adding a definite and constant quantity of water to the dry material in the dilatometer, it was essential that the finely divided substance should be readily wetted in a uniform manner. Gelatin behaves satisfactorily when placed in cold water but most other dry hydrophilic substances tend to form a lump, the interior of which is only very slowly wetted.

After the initial swelling of the gelatin in cold water had taken place, the dilatometer could be immersed in warm water, this causing the formation of a sol which upon cooling would set to a gel.

The other substance chosen as an example of the elastic gel type was the thick portion of the white from fresh eggs.

Materials of the inelastic-gel type were activated silica gel obtained from the Silica Gel Corporation, and the colloidal coagulum of ferric hydroxide.

Experimental Data

The Volume Change of Toluene with Temperature.—In order to test the accuracy of the dilatometer, a study was made of the behavior of pure toluene upon exposure to temperature changes. The dilatometer was completely filled with toluene, and the readings taken at different temperatures were plotted. The resulting curve is shown in Fig. 2. All readings fell on a straight line and the values were easily reproduced in repeated temperature-lowering or temperature-raising cycles. When this line was checked against the existing data on the contraction of toluene²⁵ our values proved to be about 2.5% too high. No reason was found for this discrepancy. Table I gives the readings from which the toluene curve was constructed.

In recording the data taken in these studies, it was considered, in every case, that the toluene column had zero length when the system was in equilibrium at 0°C. Therefore the readings which resulted from temperature-lowering are recorded as cm. contraction or as a negative length of the toluene column. If then at any temperature an expansion occurred which caused the toluene column to rise above the point at which it stood at 0°C, the readings became positive in sign. It will be seen that this change in sign of toluene length occurred in every case where water was frozen.

The Volume Change of the System, Toluene-Water, with Temperature Change.—Fig. 2 shows the form of the curve which results from freezing a sample of pure water. In this instance 1.955 grams of distilled water was frozen. The line AB represents the contraction of the toluene-water system between the temperatures 0°C and -11.1°C; line BC represents the elongation of the toluene column in the capillary stem of the dilatometer due to expansion of the system produced by separation of ice at a constant temperature; line CD then represents the contraction of the system, toluene-ice. It will be observed that the curve resulting from the freezing of the water sample is well represented by a straight line, as is indicated by readings taken both during temperature-lowering and temperature-raising.

Fig. 2 represents but one of many determinations, all of which were in exceedingly close agreement. Accordingly, the curves of Fig. 2 have been used as reference curves in later studies.

In most of the experiments undertaken a constant quantity of water was used, the variable factor being different materials under investigation of varying dry weights of samples of such materials mixed with the given weight of water.

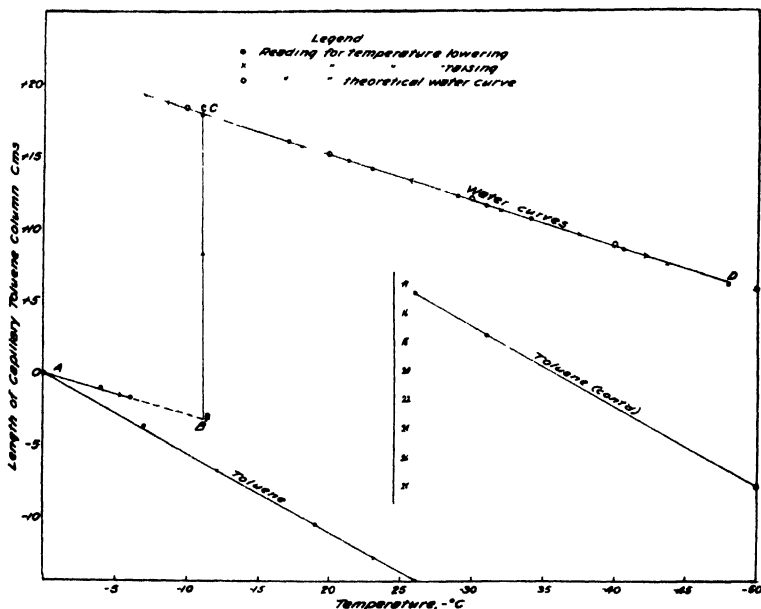


FIG. 2

Upper curve: Reference water-curve for 1.9555 grams distilled water.

Lower curve: Observed volume change of 4.2836 grams toluene upon exposure to temperature change.

However, as later results will indicate, it became necessary to calculate the "water curve" one might expect with any given quantity of water. Also, it was desirable to correct the water curve shown in Fig. 2 in certain instances when relatively large quantities of solid materials were present in the system.

In order to make such calculations, it was assumed that the slope of the toluene-ice curve was determined almost entirely by the amount of toluene which was present. Therefore the quantity of toluene remaining in the dilatometer after the expansion produced by freezing would be directly related to the contraction which would result from lowering the temperature of the system. Fig. 2 shows the agreement which was found between the calculated water curve and the measured water curve, the calculated curve coinciding almost exactly with the experimental curve. Therefore, in later experiments a theoretical water curve was utilized, and in certain experiments corrections were made for the volume of the toluene displaced by the dry sample added to

the toluene-water system. Where these corrections were made, the value taken for the thermal expansion of toluene was that observed in these studies, rather than the theoretical coefficient of expansion of toluene.²⁵

In Table II are given the readings from which the experimental water curve and the theoretical water curve were constructed.

TABLE I
Volume Change of Toluene with Temperature Change

Observed Temperature °C	Contraction of Capillary Toluene Column cm.
0.0	0.00
- 7.0	3.65
-19.0	10.50
-26.0	14.40
-31.0	17.25
-23.1	12.80
-12.1	6.60

TABLE II
The Volume Change of the System Toluene-water with Temperature Change
(1.9555 grams water, 4.2838 grams toluene)

Treatment and Process	Experimental Water Curve		Calculated Water Curve	
	Temperature °C	Length of Capillary Toluene Column cm.	Temperature °C	Theoretical Length of Capillary Toluene Column cm.
1st freezing	0.0	0.00	-10.0	+18.40
	-11.1	+17.90	-20.0	+15.25
	-17.1	+16.10	-30.0	+12.20
	-23.0	+14.20	-40.0	+ 9.10
	-29.0	+12.35	-50.0	+ 6.00
	-31.0	+11.70		
	-34.1	+10.80		
	-40.6	+ 8.75		
	-48.0	+ 6.30		
	-43.7	+ 7.70		
	-39.9	+ 8.95		
	-37.5	+ 9.75		
	-32.0	+11.35		
	-18.0	+15.75		
2nd freezing	- 7.0	+19.25		
	0.0	0.00		
	-16.1	+16.30		
	-11.1	+17.85		
	- 5.0	+19.75		

As already noted, the density change which results when 1 gram of water freezes, causes an increase in volume of 0.090740 cc.²⁵ In the two determinations tabulated in Table II the expansion indicated a volume change of 0.094020 and 0.094025 cc. per gram of water. Again no cause can be assigned to this discrepancy. In no determination which we have made on the freezing of pure water was the theoretical value for expansion due to crystallization obtained. Accordingly, in the theoretical water curves constructed for our experiments we have taken our experimental value for the coefficient of expansion of water upon freezing.

Studies on gelatin.—The gelatin used in the following studies was Difco Standardized Bacto-gelatin. The product was not further purified. The samples were of air-dry granular gelatin taken from a tightly stoppered container. It was used air-dry, since oven-drying was found to produce a marked effect on the behavior of water-binding.

The dilatometer was filled as follows: The dry, granular sample of gelatin of the desired weight was first placed in the well-dried dilatometer bulb. The desired quantity of water was then pipetted into the dilatometer bulb and toluene was immediately added so as to preclude loss of water through evaporation. Toluene was added until the bulb was practically full, at which time the ground-glass stopper was tightly fitted into place and firmly secured. A very thin film of stop-cock lubricant was found to be of value in preventing leakage of the material in the dilatometer. By this method of filling, a small bubble of air would remain in the dilatometer, but the air was readily removed by passing a very fine wire through the capillary stem into the bulb. Then by holding the stem upright and exposing the dilatometer to small temperature fluctuations, the air was removed in a series of small bubbles. If the dilatometer contained an insufficient quantity of toluene, more was added by permitting the liquid to run down the fine wire into the capillary or bulb until the desired quantity was present.

The most serious obstacle encountered in these dilatometric studies was the appearance of an appreciable quantity of air during the process of freezing. This air was not held mechanically in the system nor was it held on the surfaces of small solid particles, for it could not be removed by reduced pressure and it appeared gradually, as a result of the freezing process. Distilled water, which had been boiled and cooled under toluene, readily "froze out" relatively large bubbles of air, which indicated that the air was held in solution. It was often necessary to repeat the freezing and thawing process from two to five times before records were taken in order to "freeze out" all of the air. The appearance of air, after freezing began, distorted the true shape of the curve, and therefore only those determinations in which no air appeared were accepted as indicating the behavior of colloidal systems upon freezing. Gelatin was found to give exactly reproducible data upon repeated freezings, which fact was accepted as evidence that the figures which are to follow represent a true picture of the freezing process.

In Fig. 3 and Table III are shown the data obtained by freezing 2.3080 grams of pure water in the dilatometer. The points when plotted with change in length of capillary toluene column as ordinates and with temperature as the axis of abscissae (as in Fig. 1), gave a straight line. When an approximately 10% gelatin gel containing the same weight of water was frozen in the same manner, an essentially straight line was again obtained. It will be noted that the readings taken during the warming of the frozen material fell, within experimental error, on the line drawn through the points given by the temperature-lowering process.

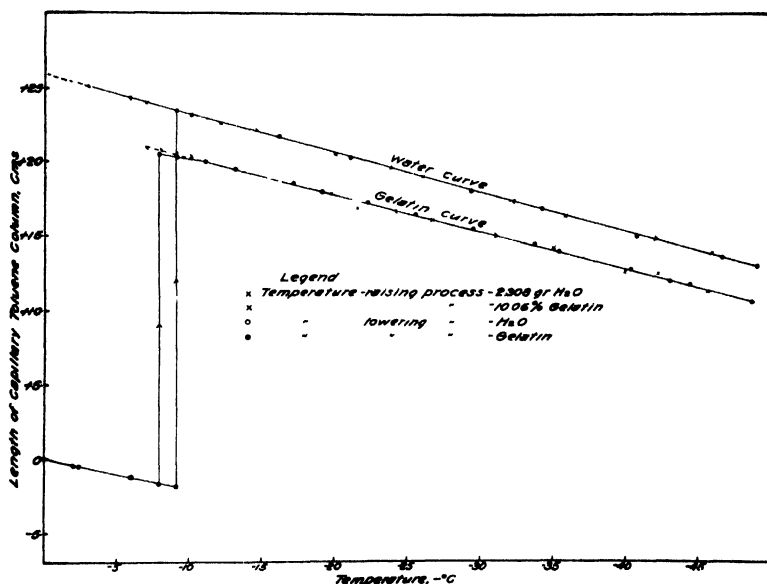


FIG. 3

The dilatometric behavior of a gelatin gel upon exposure to low temperatures.

Conclusions drawn by other workers^{2,12,36,56} indicated that cooling the system gradually to lower and lower temperatures should cause a gradual and progressive freezing of the water in the system and that the resulting curve would resemble in general form the shape of vapor pressure curves obtained when similar materials are desiccated.

Fig. 3 shows that, almost within the limits of experimental error, the points of the temperature-raising curve fall on those of the temperature-lowering curve. Also that practically no change was found in the quantity of water which will freeze beyond the point at which initial freezing took place (-8.0°C) even though the temperature was dropped to -48.6°C .

Table IV and Fig. 4 show the effect of repeated freezing upon a gelatin gel. The gelatin curve is the result of two consecutive freezings, the first temperature lowering being to -48.6°C , the system then being raised to -6.0°C , and again lowered to approximately -60.0°C . Agreement between

TABLE III

Showing the Data obtained by freezing 2.3080 grams of Distilled Water and a 10.06% Gelatin Gel containing 2.3080 grams of Water

Distilled Water		Gelatin Gel	
Observed Temperature	Length of Capillary Toluene Column	Observed Temperature	Length of Capillary Toluene Column
°C	cm.	°C	cm.
0.0	0.0	0.0	0.0
- 2.4	- 0.5	- 2.0	- 0.45
- 6.0	- 1.2	- 6.0	- 1.30
- 9.1	- 1.8	- 7.9	- 1.70
- 9.1	+23.5	- 7.9	+20.50
-10.1	+23.2	- 9.1	+20.30
-16.1	+21.7	-11.1	+20.00
-21.0	+20.3	-13.1	+19.50
-29.3	+18.1	-17.1	+18.60
-34.2	+16.9	-19.0	+18.00
-41.7	+15.0	-22.2	+17.30
-46.6	+13.6	-25.5	+16.50
-49.0	+13.0	-29.5	+15.50
-45.9	+13.9	-33.7	+14.50
-40.7	+15.1	-35.4	+14.00
-35.8	+16.4	-40.3	+12.80
-26.0	+19.0	-43.0	+12.00
-20.0	+20.5	-44.4	+11.80
-14.1	+22.1	-48.6	+10.60
-12.1	+22.6	-45.6	+11.30
- 7.0	+24.0	-42.2	+12.60
- 3.0	+25.1	-40.7	+13.10
		-35.0	+14.20
		-29.2	+15.00
		-26.7	+16.10
		-21.5	+16.90
		-19.7	+17.90
		-11.1	+20.10
		-10.1	+20.40
		- 9.1	+20.60
		- 8.0	+20.80
		- 7.0	+20.90

the points representing each determination could hardly be closer, even though in the second case the temperature decrease was 20% greater than in the first. Particular attention is called to the fact that as shown by Fig. 4 the temperature-lowering and temperature-raising processes do not result in coincident lines. A consideration of this fact is not imperative at this time; rather, it is important that each line is the result of plotting the closely agreeing readings from two separate determinations.

In studying a system as concentrated as that reported in Table IV, it was found necessary in order to obtain a homogeneous gel to immerse the dilatometer in warm water and convert the mixture into a sol, which then was allowed to set to a gel. Accordingly, it was deemed advisable to ascertain whether or not the temperature at which peptization took place had any effect upon the subsequent freezing behavior of the system. Table V and Fig. 5

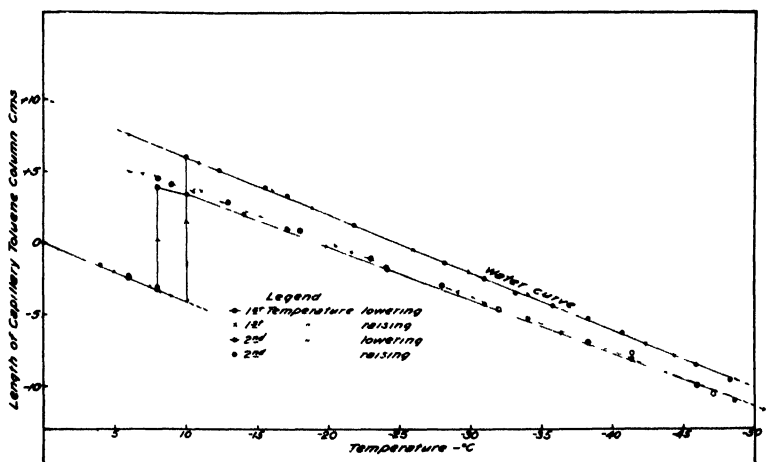


FIG. 4

The effect of repeated freezing upon the dilatometric behavior of a gelatin gel.

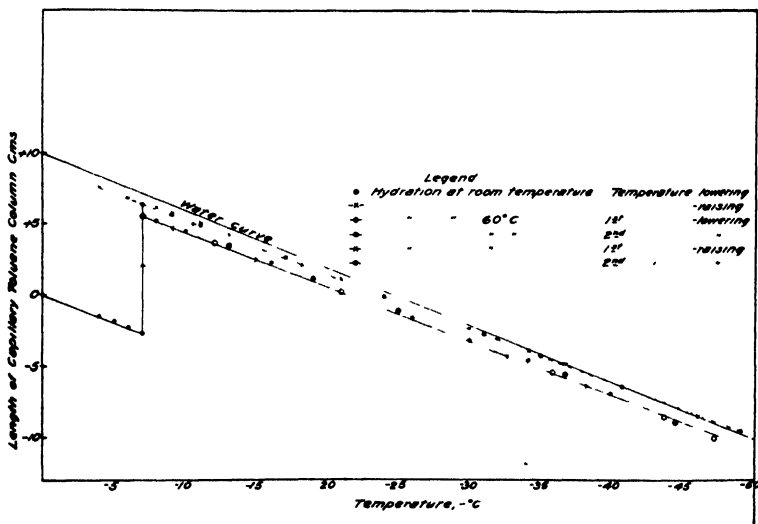


FIG. 5

The effect of the temperature of hydration upon the dilatometric behavior of a dilute gelatin gel upon exposure to low temperature.

TABLE IV

Showing the Data obtained by freezing 0.9300 gram of Distilled Water and a 50% Gelatin Gel containing 0.9300 gram of Water.

Distilled Water		Treatment and Process	0.4650 gm. gelatin + 0.9300 gm. H ₂ O	
Observed Temperature	Length of Capillary Toluene Column		Observed Temperature	Length of Capillary Toluene Column
°C	cm.		°C	cm.
0.0	0.00	1st freezing	0.0	0.00
- 5.0	- 2.00		- 4.0	- 1.55
- 7.5	- 3.05		- 6.0	- 2.25
- 8.2	- 3.35		- 8.0	- 3.05
- 9.0	- 3.70		- 8.0	+ 3.85
- 10.1	- 4.00		- 10.1	+ 3.40
- 10.1	+ 6.00		- 14.1	+ 2.00
- 12.4	+ 5.05		- 17.1	+ 1.00
- 15.6	+ 3.85		- 31.0	- 4.25
- 17.1	+ 3.25		- 34.1	- 5.35
- 21.8	+ 1.25		- 41.4	- 8.05
- 28.2	- 1.40		- 48.6	- 11.05
- 31.0	- 2.50		- 41.4	- 8.10
- 33.2	- 3.40		- 34.1	- 5.25
- 35.8	- 4.40		- 21.0	- 0.20
- 38.3	- 5.30		- 11.1	+ 3.60
- 40.7	- 6.30		- 9.1	+ 4.20
- 45.9	- 8.50		- 8.0	+ 4.55
- 48.2	- 9.65		- 7.0	+ 4.80
- 44.4	- 7.90		- 6.0	+ 4.95
- 34.1	- 3.60	Thawed and re-frozen		
- 26.0	- 0.50			
- 16.1	+ 3.65	2nd freezing	- 6.0	- 2.45
- 6.0	+ 7.55		- 8.0	- 3.20
			- 8.0	+ 3.85
			- 17.1	+ 0.85
			- 24.0	- 1.70
			- 32.0	- 4.65
			- 41.4	- 7.65
			- 47.1	- 10.60
			- 60.0*	- 14.25
			- 45.9	- 9.95
			- 38.3	- 6.85
			- 28.0	- 2.95
			- 23.0	- 1.05
			- 18.0	+ 0.85
			- 13.1	+ 2.70
			- 9.1	+ 4.05
			- 8.0	+ 4.65

*(approximated)

show data resulting from such a study. In Fig. 5 the water curve for 0.9300 gram water from Fig. 4 has been drawn as a reference line. In this experiment 0.1000 grams of dry gelatin was added to 0.9300 grams of cold water, and the gelatin was allowed to swell for eight hours. The dotted line in Fig. 5 represents the curve obtained upon freezing the mixture. The gelatin curve gradually approaches the water curve, actually crossing it at the very low temperatures. This crossing may be explained by the fact that the water curve has not been corrected for the presence of the dry sample. It should be noted that there is no indication of gel alteration, since temperature-lowering and temperature-raising processes result in an identical line.

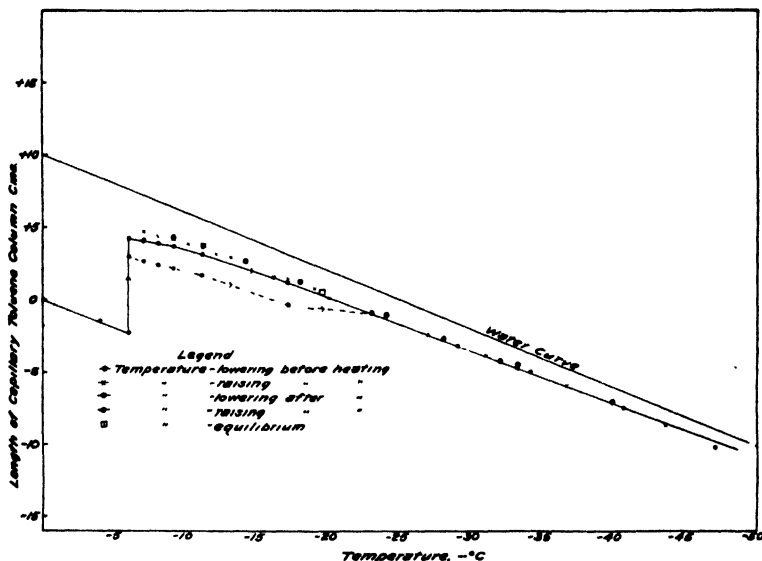


FIG. 6

The rate of establishment of equilibrium of ice formation in a 50% gelatin gel at different low temperatures with reference to the effect of elapsed time upon the subsequent behavior of the gelatin gel upon freezing.

The lower line of Fig. 5 is the plot of the data resulting from two consecutive freezings of the system described above after the hydrated gelatin has been converted to a sol by warming at a temperature of 60°C. Again, it is considered that no alteration was effected by freezing, as most of the readings from the two freezing processes lie on the same straight line.

It is evident from an inspection of the data in Table V and the graphs in Fig. 5 that the temperature at which gelatin is peptized plays a rôle in determining the water-binding capacity of a gelatin-gel as measured by dilatometric methods. The difference in water binding probably is the result of a more complete hydration of the ultimate gelatin particles when peptized at the higher temperatures. One may picture the gelatin hydrated at room temperatures as consisting of particles enveloped by thick shells of adsorbed water, with many particles still retaining their individuality as completely as

TABLE V
Showing the Effect of the Temperature at which Gelatin is peptized upon the Quantity of Water which remains unfrozen in a System of 0.1000 gm. Gelatin + 0.9300 gm. H₂O

Treatment and Process	Observed Temperature °C	Length of Capillary Toluene Column cm.	Treatment and Process	Observed Temperature °C	Length of Capillary Toluene Column cm.
Gelatin swelled 8 hours at room temperature, previous to freezing	0.0	0.00	Same sample warmed at approximately 60°C to form sol	0.0	0.00
	-5.0	-1.85		-4.0	-1.50
	-6.0	-2.25		-6.0	-2.25
	-7.0	-2.70		-7.0	-2.65
	-7.0	+6.35		-7.0	+5.55
	-9.1	+5.65		-8.0	+5.20
	-11.1	+4.90		-10.1	+4.50
	-17.1	+2.65		-16.1	+2.25
	-24.0	-0.10		-26.0	-1.60
	-31.0	-2.70		-30.0	-3.20
1st freezing	-35.0	-4.25	Same sample warmed to -1°C Re-frozen 2nd freezing	-34.1	-4.55
	-40.7	-6.45		-39.9	-6.95
	-48.0	-9.55			
	-60.0 (approx.)	-14.85			
	-45.9	-8.50		-7.0	+5.55
	-36.7	-4.80		-12.1	+3.65
	-30.0	-2.35		-21.0	+0.25
	-21.0	+1.15		-35.8	-5.40
	-13.1	+4.20		-43.6	-8.55
	-11.1	+4.95		-47.1	-10.05
	-9.1	+5.70		-44.4	-8.90
	-8.0	+6.15		-36.7	-5.55
	-6.0	+6.85		-25.0	-1.10
	-4.0	+7.55		-19.0	+1.15
				-13.1	+3.45

Table VI presents data from a study of the effect of aging upon the behavior of a gelatin gel when frozen and also a study of the length of time which is required to establish an equilibrium in the freezing process. A part of the data in Table VI is graphed in Fig. 6.

The system was set up by adding 0.4650 grams of dry gelatin to 0.9300 grams of water and permitting it to swell at room temperature for three or four hours. The first freezing study is represented in Fig. 6 by the black dots (temperature-lowering) and by crosses (temperature-raising). A second freezing study was made after this system had stood at room temperature for 15 hours. The results of the second freezing are not plotted on the graph, but all points fell on the previously determined line. The system was then permitted to stand for five days at room temperature and when re-frozen at -11.1°C gave the same dilatometer reading as was obtained at this temperature for the first freezing. The system was now warmed in a water bath to 50°C for 20 minutes, resulting in the formation of a very viscous sol which upon cooling set to a gel. After standing at room temperature for 25 hours the system was again frozen. The temperature-lowering readings are represented in Fig 6 by the black circles and the temperature-raising curve by a cross within a circle.

It will be seen that equilibrium was apparently established at a much lower value of indicated free-water at temperatures from -6°C to -23°C than in the previous determinations. At temperatures lower than -23°C the readings all fell on the previously observed line. The temperature-raising curve coincides with the values previously obtained for the temperature-raising process.

In this instance an apparently decided effect was produced by the initiation of the crystallization process, as the readings between -6°C and -23°C differ so greatly in the temperature-lowering and temperature-raising processes.

With more dilute gelatin gels, Figs. 3 and 5, the temperature-raising and temperature-lowering curves coincided. Therefore it was assumed that some factor accompanying the increased concentration of the gelatin must be responsible for the lack of coincidence of the two curves in Fig. 6.

A pronounced retardation of the velocity of ice crystal formation resulted when any system in which material was dispersed in water was frozen. It was also apparent that the greater the concentration of the dispersed material, the greater was this retardation effect. This retardation of velocity of ice crystal formation has been observed with molecularly dispersed substances by Walton and Brann.⁶¹ The velocity changes observed in our study have been similar to those reported by Callow⁷ in a detailed study of the rate of ice crystal growth in super-cooled gelatin gels. Therefore it seemed probable that as the point of equilibrium was approached, crystal formation proceeded at such a slow rate that the small changes were not measurable in the time which we selected for observation.

In order to test this point, the system previously studied was thawed and re-frozen by holding it for a period of two hours at -18°C . The reading indicated that equilibrium had been established, as was shown by comparison

TABLE VI

A Study of the Effect of Time, Temperature and Repeated Freezings upon the Behavior of a 50% Gelatin Gel and upon the Establishment of Equilibrium in a Gelatin System—using Dilatometric Technic. - Gelatin 0.4650 gram, Water 0.9300 gram

Treatment and Process	Observed Temperature	Length of Capillary Toluene Column
First freezing	0 0	0.00
Gelatin swollen at room temperature	- 4.0	-1.45
3-4 hrs.	- 6.0	-2.25
	- 6.0	+4.25
	- 7.0	+4.10
	- 8.0	+3.90
	- 9.1	+3.70
	-11.1	+3.15
	-16.1	+1.55
	-24.0	-1.25
	-34.1	-4.95
	-29.0	-3.15
	-40 7	-7.45
	-47.1	-10.15
	-43.6	-8.65
	-36.7	-5.95
	-31.0	-3.85
	-19.0	+0.75
	-13.1	+2.95
	-11.1	+3.60
	- 9.1	+4.20
	- 7 0	+4.70
Above sample thawed and kept at room temperature for 15 hours	0.0	0.00
Re-frozen	- 6.0	-2.30
and freezing	- 7.0	-2.65
	- 7.0	+4.15
	- 9.1	+3.70
	-11.1	+3.20
	-17.1	+1.20
	-33.2	-4.70
	-24.0	-1.25
	-17.1	+1.45
	-12.1	+3.20
	-10.1	+3.85
	- 8.0	+4.40

TABLE VI (Continued)

A Study of the Effect of Time, Temperature and Repeated Freezings upon the Behavior of a 50% Gelatin Gel and upon the Establishment of Equilibrium in a Gelatin System—using Dilatometric Technic. Gelatin 0.4650 gram, Water 0.9300 gram

Treatment and Process	Observed Temperature	Length of Capillary Toluene Column
Above sample thawed and kept at room temperature for 5 days	0.0 -11.1	0.00 +3.15
3rd freezing		
Above sample thawed and held at 50.0°C for 20 minutes	0.0 -5.0	0.00 -1.90
Stood at room temperature for 25 hours	-6.0 -6.0	-2.20 +3.00
Re-frozen	-7.0	+2.70
4th freezing	-8.0 -9.1 -11.1 -17.1 -23.0 -28.0 -32.0 -39.9 -33.2 -24.0 -18.0 -14.1 -9.1	+2.45 +2.20 +1.70 -0.35 -0.90 -2.70 -4.20 -7.05 -4.45 -1.05 +1.25 +2.70 +4.25
Sample from 4th freezing thawed and re-frozen	0.0 -7.0	0.00 -2.60
5th freezing	-8.0 -8.0 -11.1 -15.2 -18.0 -21.0 -23.0 -27.0 -31.0 -31.0 -41.4 -35.8 -26.0 -16.1 -10.1	-3.00 +2.55 +1.90 +1.20 +0.20 -0.80 -1.45 -2.85 -4.35 -4.25 -8.20 -6.00 -2.30 +1.60 +3.65

TABLE VI (Continued)

A Study of the Effect of Time, Temperature and Repeated Freezings upon the Behavior of a 50% Gelatin Gel and upon the Establishment of Equilibrium in a Gelatin System—using Dilatometric Technic. Gelatin 0.4650 gram, Water 0.9300 gram

Treatment and Process	Observed Temperature	Length of Capillary Toluene Column
Above sample thawed at room temperature and re-frozen	-19.0	-0.60
6th freezing		
Held at -15.0° to -18.0°C overnight		
Above sample melted at 50.0°C and re-frozen. Held 2 hours at -18°C	0 0	0.00
7th freezing	-19.5	-0.50
	-15.2	-2.25
	-12.1	-3.30
	-9.1	-4.05
Above sample melted at room temperature	-21.0	-0.10
Frozen and held 1 hour at -21°C	-16.1	+1.85
8th freezing	-11.1	+3.50
	-9.1	+4.05
Above sample held at room temperature overnight		
To test time factor for equilibrium		
9th freezing		
Frozen and held at -11°C for:		
1.5 hours	-11.1	not in equilibrium
3.25 "	-11.1	+2.35
5.25 "	-11.1	+2.60
15.50 "	-11.1	+2.60
Above sample cooled gradually from -11.0°C to -18.0°C over period of 7 hours	-20.0	0.00
	-15.2	+1.90
Held at -19.0°C 13 hours	-11.1	+3.30
10th freezing	-9.1	+3.95

before they were hydrated, whereas if the somewhat hydrated mixture of gelatin and water is warmed to the point at which a sol is formed and is then permitted to cool, a jelly results which from all visible appearance is homogeneous. Presumably the particles in this gel present a greater area of interface to the water phase than does the system where only swelling has taken place.

with the previously determined values for -19.5°C , after the system had been subjected to a temperature as low as -40°C . In a repetition of the thawing and freezing process a period of 1 hour at -21°C was found to give an equilibrium reading. However, on re-thawing and re-freezing at a temperature of -11.1°C , equilibrium was established only after a lapse of more than three hours.

These data are taken as evidence that the great difference observed between the temperature-lowering and -raising processes in the particular case represented in Fig. 6 (Table VI—4th and 5th freezings) is partially the result of lack of attainment of equilibrium in the crystallization process.

This may further explain the almost horizontal shape of the temperature-lowering curve (black dots) between the temperatures -6°C and -11°C in Fig. 6 and the similar flattening of the temperature-lowering curve of Fig. 4 between the temperatures -8.0°C to -10.00°C . It is probably also the reason why in both Figs. 4 and 6 the temperature-raising curve lies slightly above the temperature-lowering curve. This difference in both cases is more marked at the temperatures nearer 0°C , which is the temperature range at which establishment of equilibrium would be slower.

Undoubtedly another factor affects the behaviour of gelatin gels upon freezing. This is indicated by the dotted line in Fig. 6, but has been observed in other experiments.

In all of our dilatometer studies we have observed that the sols, gels, or even pure water would readily undercool to a temperature of from -6.0°C to -9.0°C before crystallization of ice began. It is possible that the size of the dilatometer and the relatively immobile condition in which the liquid was held was responsible for this great under-cooling. In one experiment, when the dilatometer was being standardized with a given quantity of distilled water, the water sample was broken into two nearly equal-sized globules, well separated from each other by a layer of toluene. Upon freezing, one globule froze at -8.0°C and the system was held at this temperature until equilibrium was established. This equilibrium represented the freezing of only one globule of water. The temperature was then slowly lowered to -11.0°C , the contraction in the system continuing at a uniform rate. The second globule of water froze sharply at -11.0°C . In this instance, the only factor involved in the establishment of a second equilibrium was the second initiation of ice crystallization. Obviously the earlier apparent equilibrium between -8.0 and -11.0°C was spurious.

The same effect is evident in the freezing of the gelatin gel represented by the dotted line in Fig. 6. Freezing began at -6.0°C . It progressed to a point at which about 50% of the water was frozen. Then for some unknown reason crystallization ceased and contraction set in with decreasing temperature. This contraction continued at a rate which indicated that no additional quantity of water was being frozen. At -17.0°C crystallization began again and a true equilibrium was established at -23.0°C . This fact was even more evident to the experimenter than is indicated by the graph.

In order that this interrupted freezing may occur, it is not necessary to have an actual division of the sample, providing the portions are separated by some non-freezing substance. With the gelatin sample involved, the system had been warmed and mixed until it was apparently uniform. The gelatin gel, resulting from cooling, formed a continuous layer across the bottom of the dilatometer. Hardy,²¹ in microscopical studies of freezing in gelatin gels, observed that the freezing process was intermittent in certain of his gels. He found that "when a pause occurs freezing starts again, not at the original face but at a new face within the gel, thus leaving the characteristic membrane of dehydrated gel behind."

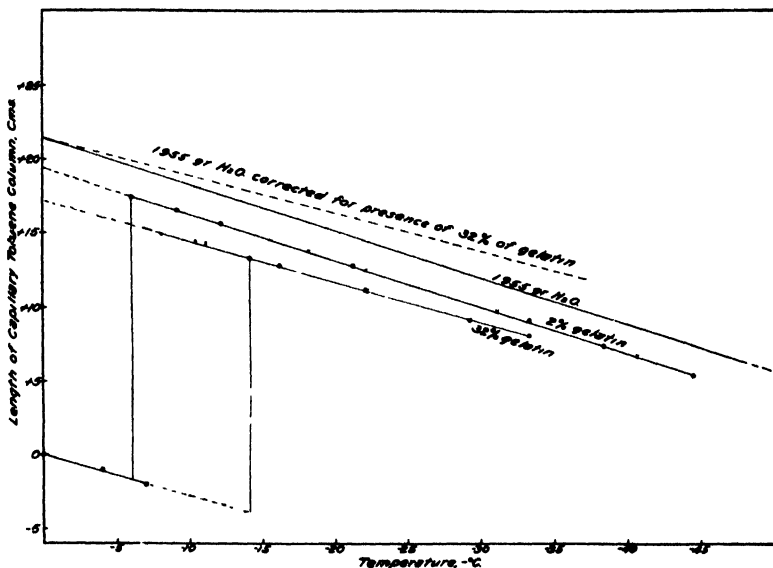


FIG. 7

The effect of concentration upon the dilatometric behavior of gelatin gel upon exposure to low temperatures.

The "intermittent freezing process" postulated by Hardy and the observed interrupted freezing noted above may be due to different causes. However, the evidence presented by Hardy demonstrates the formation of an actual boundary between a freezing portion and yet unfrozen portions of the same sample. Hardy also pointed out that in certain phases of intermittent freezing the process occurs "too slowly to be followed." Thus it may be that the intermittent process proceeds at an irregular or interrupted rate.

The significance of an interrupted freezing process in elastic gels cannot be overlooked when one is studying frozen samples by physico-chemical technic. It must also be considered in determining the temperature at which a sample should be frozen.

Fig. 6 shows that maximum freezing occurs at a temperature not lower than -11.1°C if sufficient time (5 hrs.) is given for the establishment of

equilibrium. Equilibrium is attained at -21.0°C in less than 1 hour, but there is no evidence that more water crystallized at -21.0°C than at -11.1°C , once equilibrium is attained.

A few experiments were made involving the behavior of gelatin gels of varying concentrations during the process of freezing. The data are presented in Table VII. Fig. 7 is a graphical presentation of the data obtained from the most dilute concentration (2%) and the highest concentration (32%).

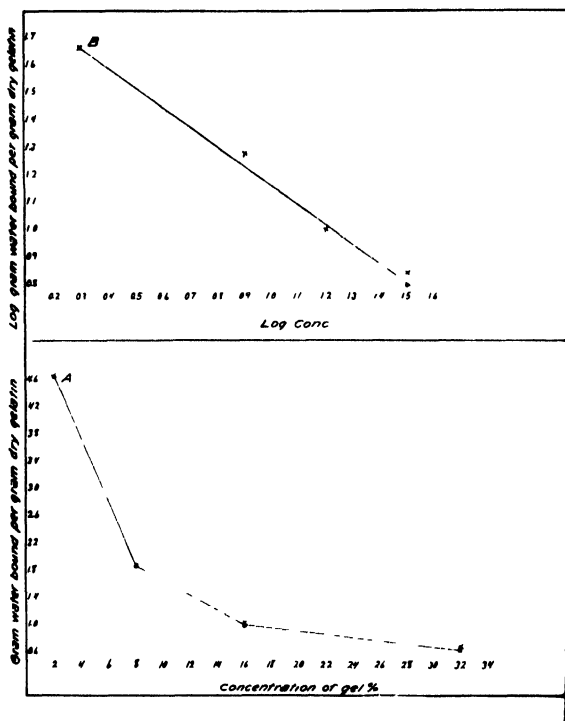


FIG. 8

Showing the weight of water bound in gelatin gels of different concentrations at the temperatures of -10.0°C and -30.0°C . Expressed as gram water bound per gram dry gelatin.

In these studies 1.955 grams of water and the desired weight of gelatin were mixed and warmed to the point at which solution was complete. With the 2% sol no correction was made for the effect of dry gelatin in the system. It will be seen that at -6.0°C , the point of initial freezing, the indicated bound-water content is 9.35%. At a temperature of -44.4°C , the bound-water content is, within experimental error of the method, exactly the same (9.35%). It is also shown that one is justified in considering the temperature-lowering and temperature-raising curve as a straight line.

The same may be said of the curve representing the freezing of the 32% gel. The behavior was observed between the temperatures of -8.0°C and -32.0°C , and all points fall easily on a straight line. If now the observed

TABLE VII

Data showing the Effect of Freezing upon Gelatin Gels of Different Concentrations— using Dilatometric Technic

2% gelatin gel		16% gelatin gel	
.0391 grms. gelatin	1.955 grms. water	.3128 grms. gelatin	1.955 grms. water
Observed Temperature °C	Length of Capillary Toluene Column cm.	Observed Temperature °C	Length of Capillary Toluene Column cm.
0.0	0.00	0.0	0.00
- 4.0	- 1.00	-11.1	+14.70
- 6.0	+17.40	-25.0	+10.70
- 9.1	+16.55	-32.0	+ 8.70
-12.1	+15.60	-39.9	+ 6.50
-21.1	+12.80	-47.2	+ 4.30
-33.2	+ 9.10	-38.3	+ 6.90
-38.3	+ 7.40	-24.0	+11.00
-44.4	+ 5.40	-16.1	+13.35
-40.6	+ 6.75	- 9.1	+15.35
-31.0	+ 9.75		
-22.0	+12.50		
-18.1	+13.80		
- 9.1	+16.60		

8% gelatin gel		32% gelatin gel	
0.1564 grms. gelatin	1.955 grms. water	0.6256 grms. gelatin	1.955 grms. water
Observed Temperature °C	Length of Capillary Toluene Column cm.	Observed Temperature °C	Length of Capillary Toluene Column cm.
0.0	0.00	0.0	0.00
- 4.0	- 1.10	-14.1	+13.35
- 7.0	+16.10	-16.1	+12.85
- 9.1	+15.55	-22.0	+11.20
-11.1	+15.00	-29.1	+ 9.15
-13.1	+14.40	-33.2	+ 8.05
-21.0	+12.00	-22.0	+11.20
-33.2	+ 8.45	-11.1	+14.30
-39.9	+ 6.50	- 8.1	+14.90
-48.0	+ 3.90		
-36.7	+ 7.35		

Thawed and re-frozen

0.0	0.00
-17.1	+13.15
-13.1	+14.35
- 9.1	+15.55

water curve be corrected for the presence of the dry gelatin (as indicated in Fig. 7 by the dotted line), the percentage of bound-water present at -8.0°C is 20.56 and at -33.0°C is 22.43. This most concentrated gelatin gel curve, then, may be considered as practically a straight line parallel to the theoretical water curve. The expression of the percentage of bound-water at the temperatures -10.0°C and -30.0°C gives an indication of the degree of parallelism between the observed gelatin curve and the theoretical water curve. Table VIII shows an actual slight decrease in total free-water content when the temperature is lowered from -10.0°C to -30.0°C . This is the reverse of what would be expected if bound water were converted into ice by a lowered temperature.

Corrections for volume of toluene displaced by the sample were based on the density of dry gelatin. Thus, knowing the weight of the dry gelatin used in the determination, the volume of toluene displaced by the gelatin could be calculated. The density of the gelatin was determined experimentally and was found to be 1.385. This method of correction is subject to question, for it is known that the volume occupied by gelatin after being wetted by water is actually less than the sum of the volume of the dry gelatin plus the volume of the water taken. Svedberg⁵⁷ found the contraction caused by wetting gelatin to be in the neighborhood of 0.055 cc. per gram of gelatin. This value is so low that this could not be the source of appreciable error under the conditions of our experiments. His study does justify the question, however, as to what may be the actual volume of the "frozen-out" gelatin.

Table VIII and Fig. 8 show the data for bound water in gelatin gels at -10°C to -30°C , as calculated from the dilatometer readings recorded in Table VII. The calculations have been expressed in the grams of water bound per gram of dry gelatin. Freundlich¹⁶ has given a general expression for an adsorption reaction which expressed mathematically is the equation for a parabola. The logarithmic expression of a parabolic curve is a straight line.

Plotting the arithmetic values of Table VIII, where the abscissa represents the grams water bound per gram dry gelatin and the ordinate represents the concentration of the gelatin gel, a smooth curve (A-Fig. 8) was obtained which appeared to be parabolic. When the logarithmic values were plotted, the resulting points fell practically on a straight line (B-Fig. 8). Accordingly it appears probable that water-binding in gelatin systems is an adsorption reaction.

TABLE VIII

The Bound Water in Gelatin Gels as a Function of Gel Concentration

Gel Concentration	Bound water expressed as per cent of total water in system		Water bound per gram dry gelatin	
	-10°C	-30°C	-10°C	-30°C
%	%	%	grams	grams
2	9.35	9.35	4.675	4.675
8	15.10	15.19	1.888	1.899
16	16.16	16.82	1.010	1.051
32	20.56	22.43	0.643	0.701

All of the studies which we have made on gelatin gels were made on gels prepared from essentially iso-electric gelatin (pH 4.7-4.8). Some experiments were made to study the effect of varying pH upon the dilatometric behavior of dilute gelatin gels when exposed to low temperature, but no observable difference due to a pH effect could be detected.

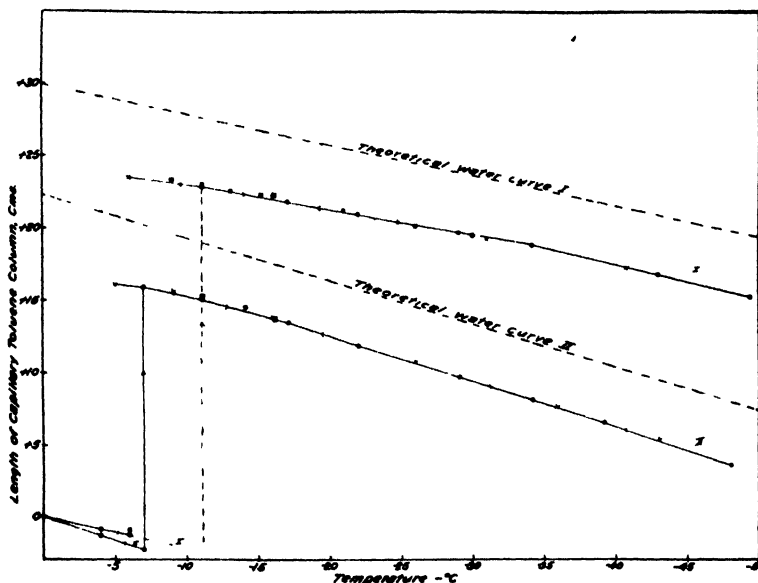


FIG. 9
The dilatometric behavior of the thick portion of egg white upon repeated exposure to low temperature.

The Behavior of the Thick Portion of Egg White from Fresh Eggs upon Freezing.—A short study was made upon the thick portion of the white of fresh eggs. This material was chosen as a natural hydrophilic colloidal system which would serve to extend the range of the bound-water studies. No attempt has been made to conduct a complete study of the behavior of egg white upon freezing.

Dilatometric analyses were made of two samples taken from two eggs not more than six hours old. The sample taken in each instance was a portion of the unmixed thick white of one egg. It was considered, as has been pointed out by St. John,⁵³ that mechanical mixing of the egg white would alter the colloidal nature of the system.

The experimental data obtained from this study are given in Table IX and Fig. 9.

These data indicate that in each sample the freezing process was completely reversible. The readings for the temperature-raising process when plotted fell on the curve representing the temperature-lowering process. Also, the data taken during a second freezing determination fell practically on the curve representing the first freezing process.

In this respect the behavior of egg white was similar to that previously observed for gelatin. However, with egg white the curves obtained were not straight lines. A more surprising fact was that they receded somewhat from the theoretical water curve at the lower temperatures. This is clearly indicated in Fig. 9 and shown numerically in Table X. The fact that the observed curve gradually receded from the theoretical curve was an indication of a contraction greater than would be expected from thermal contraction alone. If some of the "bound" water had frozen we should expect an expansion.

Egg white cannot be considered a chemical entity but is a colloidal system containing egg albumin and other proteins.

TABLE IX

A Dilatometric Analysis of the Effect of Freezing of the Thick White of Fresh Hen's Eggs

Sample 1		
Weight 3.1929 grams. Water 85.68%		
	Observed Temperature °C	Length of Capillary Toluene Column cm.
1st freezing	0.0	0.00
	— 4.0	— 0.85
	— 6.0	— 1.25
	— 9.1	—
	— 11.1	+22.85
	— 13.1	+22.55
	— 17.1	+21.85
	— 22.0	+21.00
	— 30.0	+19.55
	— 34.1	+18.90
	— 43.0	+16.80
	— 49.4	+15.25
	— 40.7	+17.25
	— 31.0	+19.30
	— 16.1	+22.30
	— 6.0	+23.50
Thawed and re-frozen 2nd freezing	— 6.0	— 0.90
	— 9.1	+23.20
	— 11.1	+22.95
	— 15.2	+22.30
	— 21.0	+21.25
	— 29.0	+19.70
	— 34.1	+18.90
	— 26.0	+20.20
	— 16.1	+22.20

TABLE IX (continued)

A Dilatometric Analysis of the Effect of Freezing of the Thick White of Fresh Hen's Eggs

Sample 2		
Weight 2.3008 grams. Water 88.53%		
	Observed Temperature °C	Length of Capillary Toluene Column cm.
1st freezing	0.0	0.00
	- 4.0	- 1.30
	- 7.0	- 2.25
	- 7.0	+15.90
	- 9.1	+15.50
	-11.1	+15.05
	-14.1	+14.50
	-17.1	+13.45
	-22.0	+11.90
	-29.0	+ 9.75
	-34.1	+ 8.20
	-39.1	+ 6.65
	-48.0	+ 3.65
	-43.0	+ 5.40
	-35.8	+ 7.70
	-26.0	+10.80
	-16.1	+13.85
	- 9.1	+15.60
	- 6.0	+16.10
Thawed and re-frozen	-16.1	+13.85
2nd freezing	-11.1	+15.20

Sörensen⁶⁵ made an extended study of crystalline egg albumin. His crystals were separated under conditions of carefully controlled concentrations of hydrogen ions and ammonium sulfate. He found that the crystals were actually hydrous egg-albumin sulfate containing 0.22 grams water per gram dry albumin.

From the fact that egg albumin may be separated in the crystalline form from a colloidal system, it seems possible that the observed greater contraction of egg white at low temperature might be due to an orientation of the hydrated egg albumin into a definite crystal lattice. This crystallization resulting from exposure to the low temperatures would take place with the formation of a more closely packed space lattice and accordingly contraction would result.

Studies on Systems of the Non-elastic-Gel Type

Studies on Activated Silica Gel.—Pulverized silica gel, obtained from the Silica Gel Corporation, was sieved; the portion which passed through the 60-mesh and was held on the 80-mesh sieve was utilized in this study. This portion was activated by heating in a vacuum oven at 150°C for 2 hours.

Systems were set up by mixing 0.4888 gram, 1.1730 grams, and 1.574 grams of activated silica gel with 1.955 grams of distilled water. Heat was liberated when the silica gel was wetted, giving evidence of a decided adsorption of

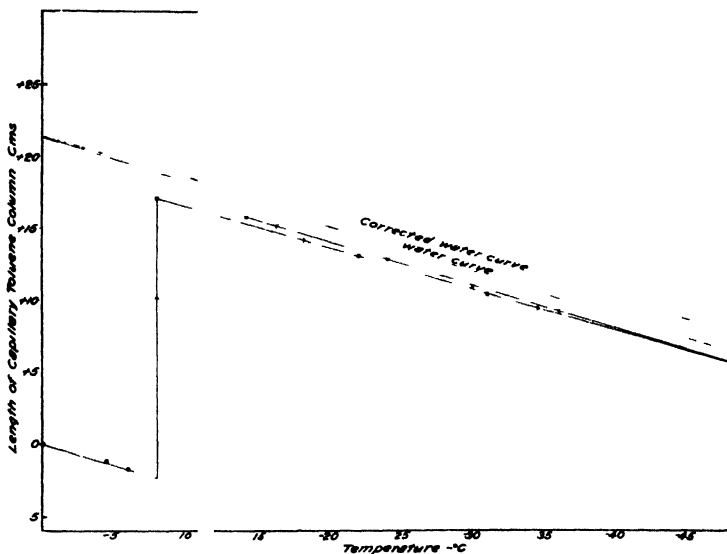


FIG. 10

The dilatometric behavior of the system Activated Silica Gel-Water upon exposure to low temperature (System consisting of 1.955 grams distilled water and 0.4888 grams Activated Silica Gel)

water. Table XI shows the dilatometric records which were made on the 25%, 60% and 80.5% silica gel systems. The data are graphed in Figs. 10, 11 and 12. In these figures correction has been made for the volume occupied by the dry silica gel, taking 2.20 as the density.

Fig. 10 shows the shape of the curve and the general agreement of experimental data obtained when the 25% mixture was frozen to -48.0°C. The points of the temperature-lowering curve fall on a straight line which is practically parallel to the corrected water curve. The line marked with the black crosses represents the temperature-raising curve. These points also fall on a straight line, but as the temperature is raised this line slowly diverges from the temperature-lowering curve.

Fig. 11 is the graphical representation of the effect of freezing upon a 60% activated silica gel system. The black dots on line AB represent the readings taken during the first freezing. The line BC represents the slope of the tem-

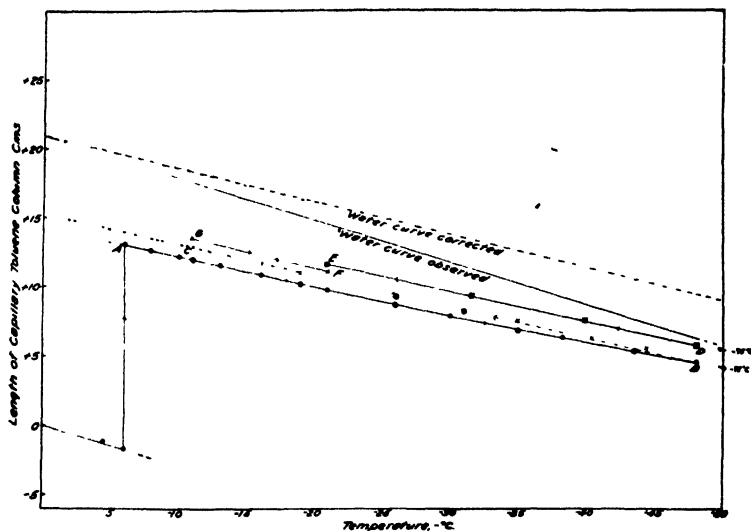


FIG. 11

The dilatometric behavior of the system Activated Silica Gel-Water upon repeated exposure to low temperature. (System consisting of 1.955 grams distilled water and 1.173 grams Activated Silica Gel).

TABLE X

A Dilatometric Study of the Thick White of Fresh Hen's Eggs. The Bound Water calculated from the Expansion which was observed due to Ice Formation and the Theoretical Expansion which should have resulted if the Total Water present had frozen

Temperature °C	"Apparent Bound-Water" at Different Temperatures Expressed as % of Total Water Present in System	
	Sample 1 %	Sample 2 %
- 6.0	17.36	20.18
- 10.0	16.19	18.16
- 20.0	14.86	17.26
- 30.0	13.36	17.71
- 40.0	13.36	18.16
- 48.0	14.36	19.73

perature-raising curve. Again, this is a straight line lying decidedly above the temperature-lowering curve.

The sample was then thawed and a second freezing was conducted. The open circles, representing these readings fall on the previously formed line. The temperature was again raised to -26.0°C and the two readings taken were found to fall on the corresponding line of the first temperature-raising process.

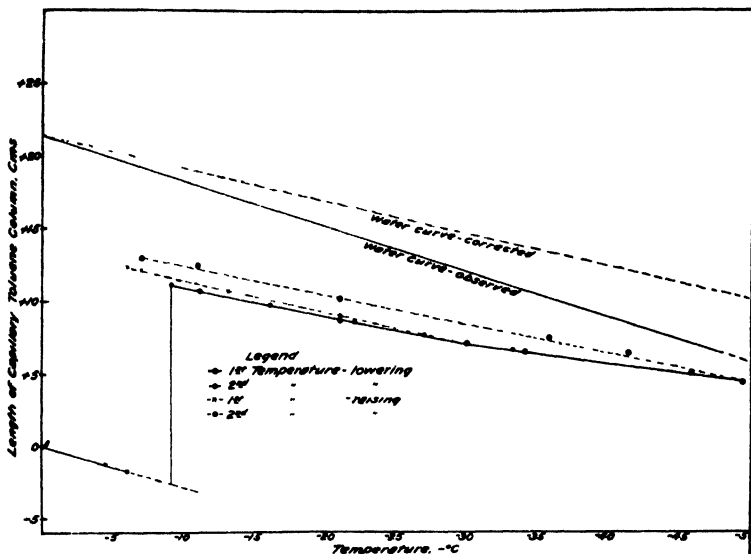


FIG. 12

The dilatometric behavior of the system Activated Silica Gel-Water upon repeated exposure to low temperatures. (System consisting of 1.955 grams distilled water and 1.574 grams Activated Silica Gel.)

Accordingly, repeated freezings gave reproducible data,* which indicated that no alteration of gel structure had resulted from the freezing process.

At this time the sample which was at a temperature of -26.0°C was cooled to an approximated temperature of -75°C . Readings were then made as the temperature was raised to -21.0°C . Line DE represents the curve obtained. Point D lies very decidedly above point B, indicating that an appreciably greater quantity of water has been frozen at -75.0°C than at -48.0°C . After warming the sample to -21.0°C (point E) it was held at this temperature for 3 hours. A reading then taken has been represented by point F. Point G indicates the reading when the sample was warmed at -11.0°C . On again lowering the temperature to -21.0°C , without further thawing the system, it was found that the equilibrium had not been disturbed.

Fig. 12 represents the behavior of the 80.5% mixture upon being frozen. The first temperature lowering was to only -33.2°C . The black dots and black

*It should be added that this statement holds when the termination of the temperature-lowering process has been at approximately the same temperature. Thus practically no hysteresis could be observed between the first and second temperature-raising processes, the termination of the first and second temperature-lowering processes being -48°C and -43.6°C respectively. Hysteresis appeared when the system was cooled to varying low temperatures. There is a decided difference in bound water values (cf. Table XII) at the same temperatures of the temperature-raising process when the termination of the temperature-lowering process has been different. Consider as an example the first and third temperature lowering processes which terminated at -48°C and -75°C respectively. With the 80% Activated Silica Gel-Water system the termination of the first and second temperature-lowering processes were -33.2°C and -49.4°C respectively. Accordingly this system likewise showed the effect of the lowest temperature reached in the freezing process. The differences in the bound water values represented at the different temperatures in Table XII are, we believe, due to the conditions mentioned above.

TABLE XI
A Dilatometric Study of the Effect of Freezing upon Systems of Activated Silica Gel and Water

System I				System II				System III			
Activated Silica		water		Activated Silica		water		Activated Silica		water	
Gel 0.4888 gram		1.9550 grams		Gel 1.1730 grams		1.9550 grams		Gel 1.5740 grams		1.9550 grams	
Treatment and Process	Observed Temperature	°C	Length of Capillary Toluene Column	Treatment and Process	Observed Temperature	°C	Length of Capillary Toluene Column	Treatment and Process	Observed Temperature	°C	Length of Capillary Toluene Column
st freezing	0.0	0.0	0.00	1st freezing	0.0	0.0	0.00	1st freezing	0.0	0.0	0.00
	- 8.0		+17.05		- 6.0		+13.00		- 9.1		+11.15
	-22.0		+13.05		- 8.0		+12.55		-11.1		+10.75
	-31.0		+10.40		-10.1		+12.10		-16.1		+ 9.75
	-48.0		+ 5.65		-13.1		+11.45		-27.0		+ 7.65
	-30.0		+10.95		-16.1		+10.80		-33.2		+ 6.65
	-24.0		+12.80		-21.0		+ 9.70		-21.0		+ 9.00
	-14.10		+15.75		-30.0		+ 7.90		-13.1		+10.70
					-38.3		+ 6.35		- 7.0		+12.10
					-43.6		+ 5.55		- 6.0		+12.35
Held at -20°C											
					-48.0		+ 4.65	for 2.5 hrs.	-22.0		+ 8.55
					-44.4		+ 5.60		-11.1		+10.95
					-41.4		+ 6.30		- 6.0		+12.10
					-35.0		+ 7.55				
					-26.0		+ 9.25	Thawed and			
					-19.0		+10.90	refrozen	-11.1		+10.70
					-11.1		+12.80	2nd freezing	-21.0		+ 8.70
									-30.0		+ 7.10

TABLE XI (Continued)

A Dilatometric Study of the Effect of Freezing upon Systems of Activated Silica Gel and Water

System II			System III		
Activated Silica Gel 1.1730 grams		water 1.9550 grams	Activated Silica Gel 1.5740 grams		water 1.9550 grams
Treatment and Process	Observed Tempera- ture	Length of Capillary Toluene Column	Treatment and Process	Observed Tempera- ture	Length of Capillary Toluene Column
	°C	cm.		°C	cm.
Thawed and refrozen 2nd freezing					
	-11.1	+11.85		-34.1	+6.50
	-19.0	+10.10		-45.9	+5.05
	-26.0	+8.65		-49.4	+4.45
	-35.0	+6.85		-41.4	+6.40
	-43.6	+5.40		-35.8	+7.45
	-31.0	+8.20		-21.0	+10.15
	-26.0	+9.25		-11.1	+12.45
	-75.0	—		-7.0	+12.90
	-48.0	+5.80			
	-39.9	+7.60			
	-31.0	+9.30			
	-21.0	+11.50			
Held 3 hours at -21.0°C					
	-21.0	+11.05			
	-11.1	+13.45			
	-21.0	+11.05			
	-11.1	+13.45			

crosses represent the readings taken during the temperature-lowering and temperature-raising processes, respectively. A second temperature lowering was made to 49.5°C. The readings of the second process lie on the previously obtained straight line to the temperature of -30.0°C. At this point, as in Fig. 11, the curve makes a decided convergence toward the water curve. The temperature-raising process from the temperature -49.5°C gives a line lying considerably above that given when the sample was frozen to only -33.2°C.

In Figs. 10, 11 and 12 the observed water curve for 1.9550 grams of water has been drawn. The water curve corrected for the volume of silica gel has been represented by a dotted line. Table XII shows the percentage of bound-water found at different temperatures, the corrected water curve being in all cases considered to represent 100% of free water.

The behavior of the activated-silica gel-water systems is very different from that of the system, gelatin-water. These differences are (A) the temperature at which maximum freezing occurred and (B) the behavior of the

frozen mass as the temperature approached the melting point of ice. In the gelatin systems maximum freezing was complete at temperatures not lower than -6.0°C and further cooling of the frozen mass to -48.0°C was without apparent effect on the bound water content of the gelatin.

With the silica gel systems gradually lowering temperatures caused increasing quantities of water to freeze; also, the temperature-raising curve lay distinctly above that for the temperature-lowering process.

TABLE XII

Showing the Apparent Per Cent of Total Water existing as Bound Water in Systems of Activated Silica Gel-Water at Different Low Temperatures

Concentration of system per cent	Process	Bound Water at Temperature Indicated				
		-10°C per cent	-20°C per cent	-30°C per cent	-40°C per cent	-48°C per cent
25	Temp. lowering	9.35	9.81	9.90	10.02	10.28
25	Temp. raising	8.01	7.94	8.64	9.30	10.28
60	Temp. lowering	29.90	28.50	27.57	25.47	22.90
60	Temp. raising, 1st	25.70	25.00	24.30	23.83	22.90
60	Temp. raising, 3d*	—	—	19.16	18.23	17.52
80	Temp. lowering	38.32	37.15	35.28	31.07	27.58
80	Temp. raising, 1st	35.75	35.75	35.28	—	—
80	Temp. raising, 2nd	31.54	30.14	28.97	27.80	26.87

* Second temperature raising not calculated.

If the behavior of inelastic gels upon freezing is analogous to that of elastic gels as indicated by gelatin, the curves representing the temperature-lowering and raising processes should be the same, and decreasing temperatures would not increase the amount of water that could be frozen.

Again, if silica gel consists of a mass possessing a structure extremely capillary in nature, as is conventionally accepted,^{10,34,46,47} it can be seen that the water in the gel must be held with a very great force of capillarity. This force is sufficiently great to resist the forces of crystallization when the temperature is lowered below the freezing point of water, keeping the water adsorbed in a liquid state. Finally, as the temperature is lowered the crystallization force becomes great enough to cause the capillary water to freeze. If the temperature be now raised a slight amount, it would seem that equilibrium should be established anew between the opposing forces of capillarity and crystal formation, with the result that some of the frozen water would melt and again become adsorbed capillary water. This would result in a completely reversible process, with the temperature-lowering and temperature-raising curves following the same path in spite of the fact that decreasing temperatures caused an increase in the quantity of water which could be frozen. That the freezing of silica gel systems is not a readily reversible process is indicated by Figs. 10, 11 and 12.

However, Figs. 11 and 12 and Table XII indicate that a partially reversible reaction takes place in the 60% and 80.5% samples. The slope of the temperature-raising curves in Figs. 11 and 12 is divergent from the corrected water curve, indicating that actually less free-water is present at -10.0°C after the sample has been warmed from -48.0°C than is present at the point of -48.0°C . The same tendency is more clearly indicated in Fig. 11 by the points E and F. E was the reading given when the frozen mass which had

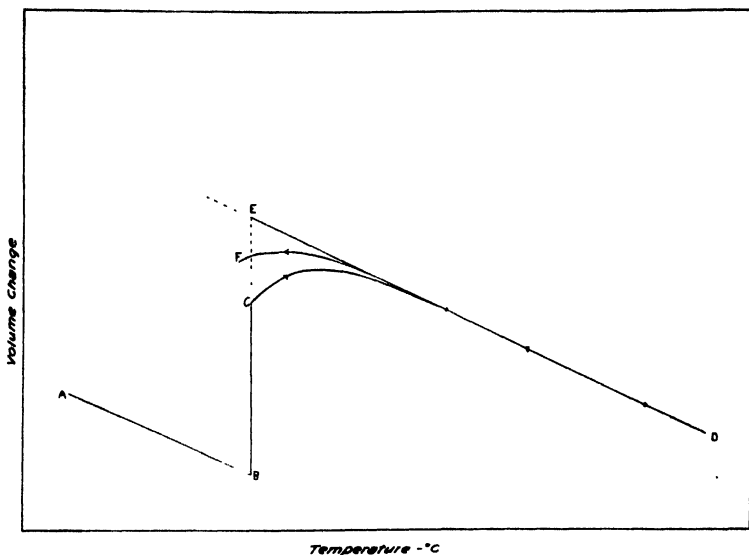


FIG. 13
The general dilatometric behavior of inorganic hydrogels upon exposure to low temperature, as observed by Foote and Saxton.

been cooled to -75.0°C was slowly and gradually warmed to -21.0°C . When the temperature was held at -21.0°C for several hours, point F was found to be the measured value for that temperature, rather than E. Evidence that this is nearer the true point of equilibrium is shown by the facts that (a) after raising the temperature to -11.0°C the same value was obtained when the temperature was again lowered to -21.0°C and (B) when the lines DE and FG are extended they are found to intersect at the 0.0°C line.

This difference between the points E and F would indicate that the velocity of equilibrium establishment is a factor determining in some degree the slope of the temperature-raising curve. Time cannot be considered the only factor, for after a period of three hours the line FG, which apparently represents the points of true equilibrium, lay distinctly above the corresponding portion of the line BC. Evidently, then, some difference was produced in the point of true equilibrium when the sample was cooled to -75.0°C rather than only to -48.0°C .

Studies on Colloidal Ferric Hydroxide Systems.—We have already noted that Foote and Saxton^{13,14,15} investigated the effect of freezing upon certain

inorganic colloidal systems. The general shape of the Foote and Saxton curve is indicated in Fig. 13. They assumed that three kinds of water existed in their gels, (A) "free-water," (B) "apparent capillary-water," (C) "combined-water." The free-water was considered to be the water which froze readily at a constant temperature. This is indicated in Fig. 13 by the vertical line BC. As the temperature was gradually lowered to point D, the curved line CD was obtained. The gradual freezing, represented by the curved line, was considered a freezing of water held in the capillary spaces of the gel. Raising the temperature from D to near the zero point gave the line DF. DE is a straight line formed by an extension of the straight portion of line DF. DE was considered the theoretical measure of the combined water. The gradual sloping away of DF from DE was considered to be due to the remelting, as the temperature approached 0.0°C , of some of the water held by capillarity. As Foote and Saxton were unable to find a point at which there appeared to be a sharp distinction between "free-water" and "apparent capillary-water" they empirically chose -6.0°C as the temperature at which all "free-water" was frozen. The vertical distance between the observed point C and the extrapolated point E was considered to be a measure of the "apparent capillary-water."

Our results are at variance with those of Foote and Saxton. We have shown that with gelatin, if sufficient time were given for equilibrium to be established at the point of initial freezing, the line CD was a straight line and the line DF was also a straight line coinciding with CD. With activated silica gel, lines CD and DF were both straight, though not coincident.

It seemed that two possible explanations might be advanced for the curved line obtained by Foote and Saxton, (A) that a true equilibrium was not established at the temperature of initial freezing (their point C) before the temperature was again lowered, or (B) that the sample, if not electrolyte-free, would at first freeze gradually and also begin to melt at temperatures lower than zero.

The following experiments were carried out on a hydrated ferric hydroxide coagulum to clarify, if possible, the points in question. The hydrated ferric hydroxide-gel was prepared as follows: 100 cc. of 30% ferric chloride solution was added to 400 cc. of boiling water. The colloidal ferric hydroxide which formed was precipitated by the addition of a sufficient amount of dilute ammonium hydroxide. The coagulum was dialyzed against distilled water until no chlorides appeared in the external liquid. The coagulum was filtered and sucked as dry as possible upon a Büchner funnel. The mass of ferric hydroxide was then well mixed and aged for several days over distilled water in a closed vessel. The resulting ferric hydroxide, although having a moisture content of from 85-88%, had the consistency and appearance of thick apple butter. Tables XIII and XIV and Fig. 14 show the data resulting from the dilatometric measurements made upon this hydrated ferric hydroxide.

System I consisted of a 3.541 gram sample containing 84.22% water. Initial freezing took place at -6.0°C , and after freezing was complete the contraction-expansion curve to -48.0°C was a straight line. As the temperature was gradually raised to -6.0°C there resulted a second straight

line, but as in the activated silica gel studies, the temperature-lowering and temperature-raising curves were divergent from the point of lowest temperature.

Determination II is a repetition of determination I. In System II the sample weighed 2.7071 grams containing 85.25% water. The solid black lines of this part of Fig. 14 represents the results of the first freezing process. In this determination, as in the former, the points of the temperature-lowering and temperature-raising process lie on straight lines diverging from the point of lowest temperature.

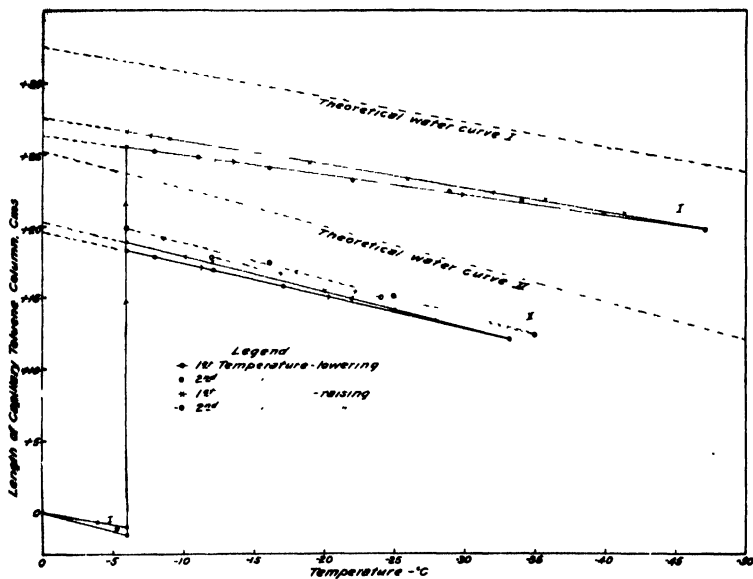


FIG. 14

The dilatometric behavior of hydrous ferric hydroxide upon exposure to low temperature.

With both samples I and II freezing was observed to result in a very decided physical alteration. When the sample was thawed, the ferric hydroxide settled in brown flakes from clear water, the water representing a large percentage of the total volume of the sample. Thus the "apple butter" consistency of the coagulum was completely disrupted. Accordingly a second freezing was made of sample II. Readings of this freezing are indicated by open circles and by a cross within a circle. The resulting lines follow closely the lines of the first freezing process.

In this study with ferric hydroxide the results coincide in many respects with those found by Foote and Saxton, with the exception that all of these readings fell on straight lines, while those of Foote and Saxton curve decidedly between the temperature -10.0°C and -6.0°C .

It was a matter of considerable surprise to observe that the ferric hydroxide, completely precipitated by the effects of the first freezing process, should continue to exhibit such great ability (Table XIV) to prevent water from freezing.

The theoretical water curves have been included in Fig. 14. It will be seen that decreasing temperatures cause a gradual approach toward this line, as would be expected from the convergence of the temperature-lowering and -raising curves.

TABLE XIII

A Dilatometric Study of the Effect of Freezing upon the State of Water in a Colloidal Ferric Hydroxide Coagulum

Sample I. 3.541 grams (water 84.22%)

Sample II. 2.7071 grams (water 85.25%)

Remarks	Observed Temperature °C	Length of Capillary Toluene Column cm.	Remarks	Observed Temperature °C	Length of Capillary Toluene Column cm.
First freezing	0.0	0.00	First freezing	0.0	0.00
— 4.0	— 0.70		— 6.0	— 1.60	
— 6.0	— 1.00		— 6.0	+18.25	
— 6.0	+25.55		— 8.0	+17.80	
— 8.0	+25.20		—12.1	+16.90	
—11.1	+24.80		—17.1	+15.75	
—16.1	+24.05		—25.0	+14.10	
—22.0	+23.20		—33.2	+12.10	
—29.0	+22.45		—28.0	+13.25	
—34.1	+21.80		—20.0	+15.45	
—39.9	+20.95		—12.1	+17.50	
—47.1	+19.80		— 6.0	+18.90	
—41.4	+20.90		—13.1	+17.50	
—35.8	+21.80		—21.0	+15.85	
—26.0	+23.30		—25.0	+14.90	
—19.0	+24.45		—15.2	+17.45	
— 9.1	+26.10				
— 6.0	+26.60		Thawed and refrozen	—12.1	+17.80
			2nd freezing	—24.0	+15.00
				—35.0	+12.45
				—25.0	+15.10
				—16.1	+17.45
				— 6.0	+19.85
			Thawed and refrozen, 3rd freezing	—12.1	+17.95
				—23.0	+15.75
				—34.1	+12.95
				—26.0	+15.05
				—16.1	+17.50
				— 6.0	+19.85
			Thawed and refrozen, 4th freezing	—12.1	+17.95
				—22.0	+15.95
				—32.0	+13.40
				—25.0	+15.20
				—16.1	+17.45
				— 6.0	+19.75

TABLE XIV

A Calculation of the Percentage of the Total Water in Colloidal Ferric Hydroxide Systems which remained unfrozen when the System was cooled to Various Temperatures. (Data calculated from Table XIII)

Sample	Remarks	Point of Initial Freezing		Temperature of Lowest Exposure	
		Temperature °C	Amount of "bound" water per cent	Temperature °C	Amount of "bound" water per cent
I	Temperature-lowering	- 6	18.07	-47	13.63
	Temperature-raising	- 6	14.85	-47	13.63
II	1st temperature-lowering	- 6	21.18	-33	17.22
	1st temperature-raising	- 6	19.00	-33	17.22
	2nd temperature-lowering	-12	17.02	-35	14.25
	2nd temperature-raising	-12	15.04	-35	14.25

Discussion

The Methods which were employed.—In our studies we have calculated a theoretical water curve, and in this calculation have ignored any contraction of ice upon subjecting it to decreasing temperatures. This was done because it was possible to so nearly reconstruct the actually observed curve representing the freezing of 1.9550 grams of pure water (Fig. 2), without a consideration of the thermal expansion of ice. If these water curves are erroneous, then the conclusions which have been drawn regarding the relation of observed volume increase to theoretical volume increase are in error. However the disregarding of any slight volume change of ice with temperature change has no influence upon the further consideration given to the studies on gelatin and activated silica gel, by the establishment of a "corrected water curve." This correction has been made by allowing for the volume of toluene displaced by the mass of the dry solid used in preparing the colloidal system. Obviously, with less toluene in the experimental system toluene-colloid-water than in the reference system toluene-water, the difference in quantity of toluene present must be considered, if the expansion or contraction of the volumes of the two systems are comparable when exposed to temperature change.

The Effect produced by Freezing upon Systems of the Elastic-Gel Type—(A) *Gelatin Gels.*—Under the experimental conditions of the present investigation, the freezing of the system gelatin-water was completely reversible. These results are at variance with those reported by Molisch,³⁶ and by Fischer and Bobertag.¹² They are also in opposition to the theoretical conclusions of

Kuhn,²⁹ and Stiles,⁵⁶ who considered that freezing would always be accompanied by a partial and a more or less permanent alteration of the original gel structure. The present study is in agreement with the results of Liesegang,³⁰ and Moran.³⁷

Stiles⁵⁶ has emphasized the importance of the rate of freezing of colloidal systems in relation to the effects produced by the freezing process. He found that with thin sheets of hydrated gelatin the water-loss upon thawing was from 4 to 6 times greater from samples which had been slowly frozen, than from similar samples which had been frozen rapidly. Moran's³⁷ studies, demonstrating internal and external centers of ice crystal formation in gelatin gels, may be considered a verification and explanation of the results reported by Stiles. With the slowly frozen samples the ice formation was to a large extent external and as a consequence re-adsorption of the water was slow. On the other hand, Hardy²¹ observed that when disseminated ice crystals formed *within* the gel, and when crystals of a solid solution of gelatin formed, the water resulting from thawing the frozen mass was immediately re-adsorbed.

Our experimental conditions favored rapid freezing of the gels. No permanent alteration of the gel has been observed, nor have studies under experimental conditions favoring slow freezing been made.

It should be stressed that when we refer to "an alteration of gel structure" it is with reference to a change in the gel structure of a behavior of the gel upon exposure to changing temperatures which is detectable and measurable by dilatometric technic. Thus in our system the gelatin gels occupied the same volume at any given temperature above 0°C after the sample had been frozen as had been occupied before freezing. Moran³⁷ believed that he could detect a slight volume change brought about by the process of freezing and thawing. Also, in our experiments the process of repeated freezing and thawing gelatin gels in no way altered the quantity of water which crystallized at the initial freezing point of the system. This has been interpreted as indicating that ice-crystallization took place relatively rapidly and that the centers of crystal formation were principally internal.

These results are considered as additional evidence in favor of the theory advanced in part by Hardy and in part by Fischer, Hardy postulating that dehydration could be considered a reversible process if a gel or sol resulted normally from the addition of a colloidal substance to water, and Fischer considering the process of freezing only a certain type of dehydration.

Moran, by means of the dilatometer, found that, with a 43.7% gelatin gel held at -11.0°C ice crystal formation was only complete after a period of twenty-six days. In view of his study it is pertinent to ask whether our results represent a true equilibrium of the freezing process. Our data designed to test this question indicate that our values cannot be greatly in error. The fact that it was possible to obtain results clearly demonstrating a reversible reaction with the elastic gels, is evidence that equilibrium must have been practically complete. Otherwise, a reading of the dilatometer at -15.0°C would have been distinctly different after exposure of the sample to a temperature of -50.0°C than before exposure to this lowered temperature.

We have already noted that our dilatometric data indicate that water-binding in gelatin gels is an adsorption reaction. Newton and Gortner⁴³ and Gortner⁴⁵ interpret water-binding in colloidal systems of gum acacia as an adsorption reaction, although this may not hold for all samples of gum acacia [cf. Newton and Martin⁴⁴]. Further evidence of the nature of water-binding in hydrophilic colloidal systems is given by Newton and Martin.⁴⁴ Their gelatin data have been recalculated and are shown in Table XV and Fig. 15.

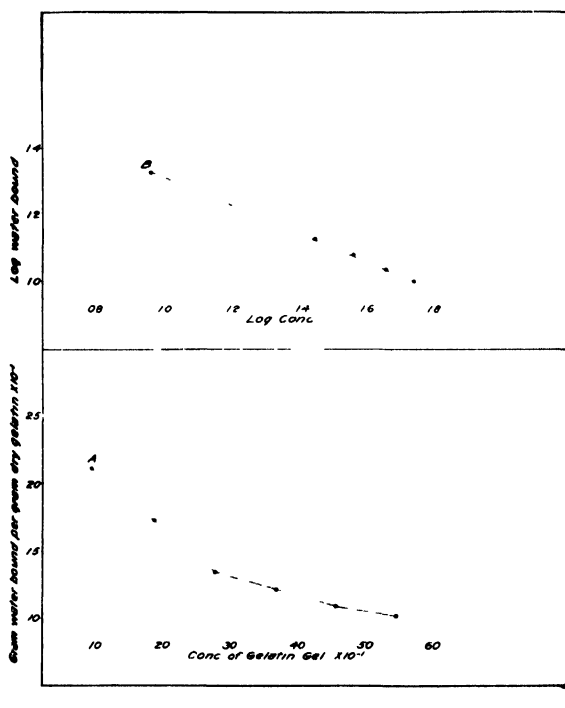


FIG. 15

Showing the weight of water bound by gelatin sols of different concentrations. Data expressed as grams water bound per gram dry gelatin (cryoscopic data of Newton and Martin).

TABLE XV

Bound Water in Gelatin Sols as a Function of the Concentration of the Gelatin Sol. Determinations by the Newton-Gortner Cryoscopic Method.

Data of Newton and Martin⁴⁴

Concentration of Gelatin in System per cent	Bound-Water per cent	Water Bound per Gram of Dry Gelatin grams
0.93	1.9	2.05
1.86	3.2	1.70
2.77	3.7	1.31
3.66	4.4	1.17
4.55	5.0	1.04
5.43	5.5	0.96

Newton and Martin's studies were carried out by the Newton-Gortner cryoscopic method, which can be used only with dilute gelatin sols, rather than gels. It will be seen that these data indicate again an adsorption reaction due to the fact that when the arithmetic values of gelatin concentration and bound water are plotted, Curve A, apparently a parabola, resulted, whereas when the logarithmic values were plotted a straight line relationship was indicated (Curve B). We have already (Fig. 8) shown a similar relationship from our dilatometric data.

A review of the literature on studies made upon aqueous gelatin-containing systems reveals a most interesting array of values for the actual quantity of water which is combined with gelatin or which exists in a "bound" condition.

The method of measurement of water-binding by gelatin has been different in nearly every study reported. Svedberg,⁵⁷ from studies upon the contraction of gelatin when wetted, considered the weight of water bound per gram dry gelatin to be in the neighborhood of 0.08 gram; Fischer, as cited by Moran,³⁷ from studies on the rate of drying found 0.1 gram water to be bound per gram dry gelatin. Taffel⁵⁸ considered the work of Sheppard and Sweet,⁵⁴ on the setting points of gels, as a basis for the value of 0.3 gram bound water per gram dry gelatin. Moran³⁷ from his dilatometric studies, found the value of 0.56 gram bound water per gram dry gelatin for gels of 43.7% and 52.1% concentration. Newton and Martin's results were obtained in a study of the freezing point depression of theoretically molar sucrose solutions.

With the technic of measurement of bound-water differing so radically, it is not surprising that different values were obtained for the quantity of water bound by a gram of gelatin. Furthermore, the concentration of the gelatin in the system is a factor which influences the value for weight of water bound per gram dry gelatin. This is clearly indicated by the results of the present study (see Table VIII and Fig. 8), and by the data of Newton and Martin (Table XV and Fig. 15). Our own data show that, in a system containing 2% gelatin at -10.0°C , 4.7 grams of water is "bound" per gram dry gelatin, while in a 32% gelatin gel at -10.0°C only 0.64 gram of water per gram dry gelatin appears to be "bound." This last value does not differ greatly from Moran's value which was also obtained by dilatometric studies.

From the standpoint that water-binding in gelatin gels appears to be an adsorption reaction, it was considered probable that the temperature at which the system would be frozen would influence the value for the quantity of water which was bound. However, no indication that temperature is a factor can be detected from an inspection of Figs. 3, 5 and 7, providing that sufficient time has elapsed for the establishment of an equilibrium in the freezing process. Over the temperature range which was studied, all of the water which could be frozen crystallized at the temperature of initial freezing and exposure of the system to decidedly lower temperatures was without effect upon the quantity of water which appeared to remain in the unfrozen condition.

A possible explanation may be that two factors are affecting the equilibrium (A) the vapor pressure of ice *decreasing* with lowering temperature and (B) an adsorption "pressure" *increasing* with lowering temperature. If the

increased adsorption tendency should be sufficient to just balance the lowered vapor pressure of the ice, then the amount of "bound" water (adsorbed water) should be a constant irrespective of temperature.

(B) *Systems of Fresh Egg White*.—With egg white, as with gelatin, the freezing process appeared to be completely reversible. St. John³⁸ made a study of bound-water in the thick portion of egg white, employing the method of Rubner³² as modified by Thoenes⁴⁰ and Robinson.⁴⁰ He found that at -5.0°C 85% of the water present appeared as bound-water, while at -15.0°C only 26% of the total water in the system appeared to be "bound." Our values on a similar system are given in Table X. It is of interest to note that while St. John's value at -5°C is greatly in excess of our values at -6°C (17.36% and 20.18%) there is much less discrepancy at -15°C . Newton and Martin⁴⁴ have also studied the question of bound water in fresh egg white, using a composite sample from 16 eggs. In their studies the whites were beaten, allowed to settle for 3.5 hours, the froth was removed, and the clear liquid underneath taken for study. This clear liquid portion was then diluted to suitable concentrations. Bound water was measured by the Newton-Gortner cryoscopic method. They found much lower values than either ourselves or St. John, *i.e.*, 0.7% bound water for a 2.37% sol, 2.3% for a 7.11% sol, and 6.5% for an 11.85% (undiluted) sol. These values (recalculated) indicate respectively 0.29, 0.30, and 0.48 gram of bound water per gram dry albumin, whereas using the dilatometric method we find (sample II at -6°C) 1.55 grams of bound water per gram dry egg white. These differences in the magnitude of the values obtained by Newton and Martin and by ourselves are too great to be explained wholly on the basis of the mechanical manipulation of the sample or from the fact that their studies were made on a mixed sample of the thin and thick portions of the white. Unquestionably the method by which bound water is determined is the primary factor. Apparently a very considerable fraction of the "bound" water as measured by dilatometric and (probably calorimetric) technics can act as a solvent for sucrose and thus lead to low values when the Newton-Gortner cryoscopic method is employed. Further studies of this colloidal system must be made before final conclusions can be drawn.

The Effect produced by freezing Systems of the Inelastic-Gel Type.—Pulverized activated silica gel proved to be very desirable material for bound-water studies. It was sufficiently granular to wet readily, was very stable in nature, and in repeated and consecutive determinations gave readily duplicable results. Thus, the water "frozen out" by temperatures decidedly lower than the initial freezing point was completely re-adsorbed when the sample was thawed. This ability to return after the freezing cycle to the condition existing before freezing occurred was not observed by us in other inelastic-gel systems. At no point in the recorded temperature study did we observe a temperature below which no more water would freeze if the temperature were lowered further. Possibly all the water added to these samples would freeze if sufficiently low temperatures were employed. If this be true, then water in the silica gel system may exist only as "free-water" and "capillary-water." All the water

which failed to freeze at the point of initial freezing might be classed as "apparent capillary-water" at this temperature. The true measure of "capillary-water" would then be all the water which did not freeze at the initial freezing point of the system. This point in the present studies appears to lie between -1.0°C and -2.0°C .

It is apparent, however, from Table XII that the system would have to be exposed to extremely low temperatures in order to freeze all of the water, since the "bound water" (*i.e.* water not frozen) values decrease very slowly with lowering temperature. Thus in the 60% gel the "bound water" has decreased only from 29.9% to 22.9% in the range -10°C to -48°C and in the 80% gel from 38.32% to 27.58% in the same temperature range. An extrapolation of the curves for "bound water" indicates that all of the water would not be frozen until the sample had been exposed to temperatures below -80°C .

Ferric hydroxide gels exhibited a behavior similar to that of silica gel. A certain portion of the total water content froze at a constant temperature and the remaining quantity of unfrozen water gradually decreased as the temperature was lowered.

Although the freshly prepared ferric hydroxide system possessed a marked degree of rigidity, the structure was readily altered by freezing. The actual affinity for water, in the ferric hydroxide coagulum, seemed to be reduced but little by freezing, but the dispersion of the particles was permanently altered. That this alteration can be the result of the disruption of capillary spaces seems unsupported by the observed behavior. If the capillaries had been so completely injured by the freezing process, it would seem that the water-binding property of the colloidal system should have been materially decreased. Apparently the freezing process has in some manner produced an agglomeration of the colloidal particles, with no great effect upon the capillarity of the substance. The first freezing cycle (Sample II) reduced the apparent "bound" water at -6°C from 21.18% to 19.00%. A second freezing cycle reduced this only slightly and two additional freezing cycles were practically without effect insofar as reducing the amount of "bound" water is concerned.

In the activated silica gel studies, no apparent disruption of the capillaries occurred, as is evidenced by the duplicability of the dilatometer readings upon repeated freezing and thawing. Foote and Saxton (13) report similar findings and note that this behavior of a silica gel system is not paralleled by the behavior of aluminum and ferric hydroxide gels.

The General Behavior of Aqueous Colloidal Systems upon Exposure to Low Temperature.—It is widely accepted that with aqueous colloidal systems there is a definite temperature, below the freezing point of the system, at which all of the "free-water" will be frozen. This conception is held especially in the field of biological investigation. With biological material this temperature has been arbitrarily selected as about -20.0°C [Rubner,⁵² Thoenes,⁶⁰ Robinson^{48,49,60}], apparently on the assumption that all of the "free" water would be frozen at this temperature and that the temperature was not sufficiently low to seriously alter the free-bound water ratio.

Our experimental data indicate that all temperatures below that of the initial freezing of systems of gelatin-water are temperatures at which all of the "free-water" but none of the "bound-water" is frozen. With the inorganic hydrogels there was no temperature within the range of 0.0°C to -50.0°C at which a lower temperature did not cause the crystallization of additional quantities of ice.

Additional studies must be carried out before generalizations can be made as to the behavior of other aqueous colloidal systems upon exposure to low temperature. It seems probable, however, that biological tissue and related systems would behave more nearly like the gelatin system than silica or ferric hydroxide gels.

The results of this study further emphasize the importance of the length of the time interval during which the freezing of a colloidal system takes place. At temperatures decidedly lower than the freezing point of the system, the gel-ice equilibrium is rapidly established. At temperatures near the freezing point of the system the slow rate of ice formation is responsible for the slow establishment of phase equilibrium.

Summary

1. Dilatometric studies have been made of the state of the water in certain aqueous colloidal systems as affected by exposure to temperatures ranging from 0.0°C to -50.0°C . The systems studied were: gelatin gels of different concentrations, the thick portion of egg white from fresh eggs, mixtures of different concentrations of activated silica gel and water, and hydrated ferric hydroxide.

The low temperatures utilized in these studies were easily produced and maintained by proper mixtures of alcohol and solid carbon dioxide. The temperatures noted were measured by means of a standardized thermometer.

2. The colloidal systems studied fall into two general classes: (a) those in which the freezing process is completely reversible; and (b) those in which the freezing is an irreversible or only partially reversible process.

3. In the studies made upon gelatin it has been found that all the water which could be frozen within the temperature range of 0.0°C to -50.0°C was frozen at the recorded initial freezing temperature (approx. -6°C) if sufficient time was permitted for establishment of equilibrium. After equilibrium establishment at this point, exposure to much lower temperatures was without effect upon the quantity of water which remained in the unfrozen condition ("bound" water).

4. The rate of establishment of ice-gel equilibrium was found to be very slow, the rate being influenced greatly by (a) the concentration of the gel, and (b) the temperature of exposure. This fact emphasizes the importance of the consideration of time as a factor influencing the quantity of water which will be frozen at any given temperature.

5. Water-binding in the system, gelatin-water, appears to be an adsorption reaction. A logarithmic relationship was found to exist between the con-

centration of the gelatin gel and the grams of water which were "bound" per gram dry gelatin. In our studies 0.70 gram of water was "bound" per gram dry gelatin in a 32% gelatin gel and 4.675 grams of water per gram dry gelatin were "bound" in a 2% gelatin gel.

6. The freezing behavior of the thick portion of fresh egg white appears to be a completely reversible process, in this respect paralleling the gelatin systems.

7. The form and the slope of the dilatometric curves indicated that no disruption of capillary spaces in silica gel occurred when silica gel-water systems were frozen. The freezing of colloidal ferric hydroxide resulted in a relatively small decrease in "capillary water."

8. The quantity of "capillary-water" which could be frozen from ferric hydroxide and activated silica gel was proportional to the temperature at which the sample was exposed. With ferric hydroxide and with 25% activated silica gel mixtures, this quantity of "capillary-water" frozen appeared to be directly proportional to the lowering of the temperature. With 60% and 80.5% mixtures of activated silica gel this relationship held approximately to temperatures as low as -30°C . At temperatures lower than -30.0°C "capillary-water" froze at a more rapid rate than at temperatures above this point.

9. It is postulated that the effect of freezing upon colloidal ferric hydroxide and similar substances is to cause in some manner an agglomeration or flocculation of the colloidal particles without materially reducing the capillarity exhibited by the substance.

10. The dilatometric method has proven a useful technic for "bound" water studies. The values for "bound" water which we have obtained in our work appear to approximate the values obtained by other workers using Rubner's (Thoenes) calorimetric method on similar materials.

11. It is emphasized that "bound" water is an indeterminate term, and that "bound" water values as experimentally determined may be expected to vary from system to system, the variation being due to many factors, not the least of which is the method selected for measurement. If biological cells and tissues are similar in their behavior to gelatin and (probably) to the thick portion of egg white, then "bound" water is a measurable entity and (using dilatometric procedure) has a constant value at least at temperatures between -6° and -50°C .

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THE STRUCTURE AND ELECTRICAL PROPERTIES OF INSULATING MATERIALS

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The importance of making available information concerning the chemical structure of commercial dielectrics is becoming increasingly evident as the mechanisms of conduction and loss of energy in them are being described. These mechanisms usually depend upon the presence of ions, either free or adsorbed, but may also sometimes depend upon the presence of polar molecules if alternating current behavior is being studied.

Important engineering studies of the electrical characteristics of solid and liquid dielectrics have been made in recent years and it can be said that in general the difficulties due to lack of exact chemical knowledge of the materials have been recognized. For example, crystals have been subjected to test because of their relative freedom from impurities, and the breakdown processes in highly purified liquids such as hexane, heptane and xylene have been carefully investigated. The highly purified liquids behave quite differently from the ordinary insulating oils in that the latter always show a residual conductivity, which, incidentally, has been traced to the presence of colloidal particles in the oils.

The belief seems to be growing that in most solid dielectrics the conduction of the electric current does not take place uniformly through the material as a whole but rather along paths of higher conductivity. In the case of crystalline dielectrics in which there are ionic conductors Smekal¹ believes that the mechanism of electrical conductance is essentially bound up with the deviation of the actual crystal structure from that of the ideal lattice. The ions which take part in conduction are assumed to be concentrated in positions in the crystal where these lattice imperfections are present and move in an adsorbed condition along the paths formed by these crystalline fissures. Smekal has estimated that the ideal part of the lattice in a crystal unit contains something of the order of magnitude 10^4 to 10^5 atoms so that the "mosaics" or blocks are truly colloidal in dimension. This idea of Smekal has found favor with many investigators who have studied the electrical properties of crystals and it is supported by others in their considerations of the thermal, optical and mechanical properties of these materials. It has been considered favorably in an interesting article by Murphy and Lowry² on the complex nature of dielectric absorption and dielectric loss.

While such a mechanism is questionable in the case of crystalline substances it appears to give a correct description of the conduction processes taking place in such moisture absorbing dielectrics as cellulose, cotton, silk, rubber, the resins, and other similar and related materials. The ability of

¹ Z. Elektrochemie, **34**, 472 (1928); Ann. Physik, (4) **83**, 1202 (1927).

² J. Phys. Chem., **34**, 598 (1930).

forms of these solids to give definite and interpretable X-ray diagrams, observations concerning the manner in which they swell in suitable solvent media, the viscosity of their solutions and their ability to form homogeneous films on water and mercury all indicate what may be termed a fiber-like structure for them.

A number of theories have been proposed to explain the mode of formation of these substances which for purposes of discussion will be classified as gels or as highly polymerized organic substances. It is more or less generally agreed that they are heterogeneous in the sense that there is present both a continuous and a disperse phase, with the two phases forming a network. Studies of their elasticity and rigidity can best be interpreted on the assumption that the disperse phase is made up of particles which have aggregated to form chains or fibrils which will be arranged regularly in some cases and randomly in others. But although the existence of these chains had been suspected for many years it is not until rather recently that their existence seems definitely established.

Discussion with regard to the actual constitution of these high molecular (or aggregate) weight compounds has centered around two theories known in the literature as the association theory of Hess and Pringsheim and the macromolecular theory of Staudinger. In the association theory smaller molecular units or residues are held together to form the aggregates through secondary valences, while in the macromolecular theory the fundamental groups are chains or fibrils of such residues which are held together by primary valence forces. Their lengths, molecular weight and other physical properties will depend upon the degree of polymerization or condensation, while their chemical properties will depend in a large measure upon the groups which happen to be present at the end of the chains. We shall favor the explanation of the macromolecular theory in this article.

It has been mentioned that the results of a number of physical studies on cellulose, silk, tissue and stretched rubber indicate the presence of long primary valence chain macromolecules. In the space available it will not be possible to completely outline these results, but the manner in which they have been obtained may be suggested. Foremost among them are the X-ray diffraction studies which have been made by Meyer and Mark, Hengstenberg, Sponsler and Dore, Herzog and Jahnke, Polanyi, Weissenberg, Clark, Hauser, and others. The point of view has gradually developed that it is not necessary, as had previously been believed, for the unit crystal cell to contain an integral number of whole molecules or its equivalent in ions. In the substances with distinctly fibrous structure the chains linked by primary valences pass through the unit cell in such a way that only two links in each chain are found in the unit crystal cell. The macromolecules themselves have been shown to be sometimes as long as 500 Å while the single molecular units from which the chain is formed will be of the order of magnitude of 10 Å in height. In cases where the chains are built in spiral form one complete turn is indicated by a distinctive periodicity in the X-ray diagram from which the height of the unit cell is defined. A certain number of these chains are held together by the secondary valence forces to form bundles or micelles. The micelles are

probably held together by means of amorphous cementing materials which would be called the dispersion medium in the language of colloid chemistry. Of course the micelles will not always be oriented with their long dimension parallel (or nearly so) to the fiber axis but it is perhaps surprising how common this arrangement is. It has been established for cellulose, silk, tissue, stretched rubber, stretched gelatin and certain of the silicates.

It is not difficult to demonstrate that the forces acting in a direction parallel to the chains are much stronger than those acting perpendicular to them. In those cases where the micelles are already oriented, the fibers subjected to tensile strength tests are very strong in the direction of the fibers and much weaker in other directions. Measurements of the coefficient of expansion in the different directions also indicate the presence of this orderly arrangement.

Parallel and random arrangements of the micelles may also be differentiated by experiments in which the swelling of the materials is studied. When a section of regular arrangement swells it expands at right angles to the direction of the fibers but is not elongated. On the other hand, a section built of micelles arranged in random fashion swells not only uniformly but also much more rapidly. In the oriented structures the micelles are more tightly packed and liquids penetrate more slowly.

One of the most difficult points in connection with these theories has been the explanation of the nature of the secondary valence forces in these high molecular weight substances. In the cases where it has been possible to obtain X-ray diagrams it appears that two carbon atoms joined by the primary valences are separated by a distance of 1.5 to 1.6 Å, and the carbon to nitrogen distance of separation is perhaps 10% less. In the case of the macromolecular theory of the composition of these materials it is assumed that the primary valence bonds act in the direction of the chains so that their strength for directions other than parallel to these chains must be explained. This may be accomplished according to Meyer and Mark³ by assuming the secondary valences to be cohesive forces of a van der Waals nature acting between atoms in different chains which will be separated by distances of the order of magnitude 4 Å. We could be more satisfied with this explanation if the mechanism of these forces could be more exactly described. The existing classical theories we owe to Debye⁴ and Keesom⁵ who have assumed them to be due to the electrostatic action of fixed dipoles or quadrupoles, and to the modification of existing dipoles by a distortion effect. In the case of interaction between fixed dipoles the forces will diminish with increasing temperature but in the case of induced dipoles the effect will be independent of temperature, according to the now well known dipole theory of Debye. The method of the theories has been to calculate the dipole and quadrupole moments from known values of the van der Waals constant. However, it appears from quantum mechanical calculations⁶ too recent to be included in the book of Meyer and Mark that

³ "Der Aufbau der hochpolymeren organischen Naturstoffe" (1930).

⁴ Physik. Z., **21**, 178 (1920); **22**, 302 (1921).

⁵ Physik. Z., **22**, 129 (1921).

⁶ Eisenschitz and London: Z. Physik, **60**, 491 (1930); London: **63**, 245 (1930).

hydrogen has a quadrupole moment which according to the classical theory would have given a value of the van der Waals constant which is much too low. Therefore, these theories are in need of some revision. The method of the new theory is to take the mutual perturbations of the periodic electronic motions into account with the result that there may be calculated both the primary and secondary valence forces for very simple molecules. The primary forces act over very short distances only (order of magnitude 1.5 \AA) but the van der Waals or secondary forces diminish with distance much less rapidly and in addition the magnitudes of the latter seem to be of the right order of magnitude. It still remains to be seen whether or not this kind of calculation can be extended to the complicated systems under discussion.

It is desired to ascertain whether or not the electrical properties of these materials will depend upon their capillary structure. The results of a number of investigations have shown that the conduction of the electric current by these substances is not due to moisture condensed on outside surfaces but takes place because of the presence of moisture and ionizable materials in them. The form of conductance vs. electrolyte content and conductance vs. moisture content curves may be considered proof of this statement. An excellent example of the effect of the presence of ionizable materials is shown in the recent studies of Kemp⁷ who has demonstrated that if rubber is purified with respect to the nitrogenous constituents always present its electrical characteristics may be considerably improved. Textile materials to be used as covering for wire are now washed in water to remove inorganic impurities.

It is suggested here that the conductance is determined by the capillary structure of the insulating materials, it being due to the ionic processes operating between individual fibers or chains. The objection may be raised that such an ionic process in conducting paths makes the presence of the complex electric currents which are always found in these substances impossible of explanation. The interionic attraction theory of electrical conductance now widely accepted indicates that free ions such as exist in the conducting paths of moisture absorbing dielectrics should behave to some extent like a dielectric, owing to the ionic atmosphere surrounding each ion. The free ions then carry not only the ordinary conduction current but also a complex current with its displacement current and conduction current components. Also in addition to the constant conduction current with its I^2R heat loss free ions may produce dielectric loss in cases where the resistance of the path is variable, and conducting paths instead of being pure resistances become equivalent to resistance in series with a condenser, giving a greater alternating current conductance. Ions, in addition to being free, may be adsorbed along the conducting paths. If these ions are at all mobile they may oscillate due to an impressed alternating field giving rise to a corresponding absorption of energy. Such a movement of ions may in certain cases be equivalent to a condenser charging and discharging current.

Any mechanism which describes the process of conduction in a dielectric must account for the fact that the conductivity is increased when the strength

⁷ Bell System Tech. J., 10, 132 (1931).

of the applied field is increased. In this case it seems reasonable to assume that the effect of increased voltage will be to increase the number of dissolved ions ~~because~~ those least strongly adsorbed will be set free to carry the current in the ordinary ways. Another possible explanation of the effect produced by the use of high field strengths may be found in the deviations from Ohm's Law first observed by Wien⁸ and explained by Joos and Blumentritt.⁹ To discuss this effect we shall have to describe the ionic atmosphere more exactly. According to the newer theories dealing with the behavior of strong electrolytes in dilute aqueous solution only ions are present, furthermore, since Coulomb's Law is assumed to express the forces between them, there will be more ions of unlike than of like sign around a given ion, in other words any given ion will in effect be surrounded by a kind of space lattice arrangement of oppositely charged ions called its ionic atmosphere. It is similar in all respects to the double layer so commonly referred to in colloid chemistry. This atmosphere has a radius which may be calculated and it requires a definite time to be either formed or destroyed. Furthermore, it will always be symmetrically built about a stationary ion. But because of its finite time of relaxation the atmosphere can no longer be built symmetrically if the ion is caused to move and it will become unsymmetrical in the direction of the motion. Before the ion there will be more ions of like charge and behind it more ions of opposite charge so that each moving ion, positive or negative, is subjected to a force which decreases its mobility. If we consider an interval of time over which the atmosphere can be regarded as relatively stationary, and if during this interval the ion is removed to a distance much greater than the thickness of the atmosphere, the influence of the latter will become small and we are left with an increased conductance because the interionic forces which decrease the ionic mobilities have been overcome.

Gemant,¹⁰ in a recent book, has suggested an explanation of the effect of high field strengths which is quite different. Accordingly to this investigator it seems more logical to assume that undissociated molecules may also take part in the conduction, their decomposition being caused by the attraction of the poles for the several parts of the molecule.

It has been noted that Smekal and others believe the conduction in crystals to be due to the presence of lattice imperfections in which the ions move in an adsorbed or free condition along paths formed by these fissures and that the Smekal point of view is supported by indirect evidences provided by the mechanical and optical properties of crystals. However, the writer believes the conductance in these systems to be a volume process of the normal ionic type in which the dielectric losses can be accounted for by the Joule heat law. This conclusion has been previously drawn by Joffé,¹¹ Phipps,¹² and by others. In the first place Joffé has shown that a crystal has a characteristic specific

⁸ Ann. Physik, (4) 83, 327 (1927); 85, 795 (1928).

⁹ Physik. Z., 28, 836 (1927).

¹⁰ "Elektrophysik der Isolierstoffe" (1930).

¹¹ "The Physics of Crystals" (1928).

¹² Phipps, Lansing and Cooke: J. Am. Chem. Soc., 48, 112 (1926).

conductance, that is, its conductance is a property of the chemical substance (KCl, SiO₂ etc.) rather than of the crystal and it is independent of crystal imperfections. This conclusion could be drawn only after the most extensive purifications of the crystals had been carried out.

According to this point of view, the electrical conductance of a solid salt depends only upon the number of free ions in the lattice at the given temperature. Furthermore, if the logarithm of the specific conductance is plotted against the reciprocal of the absolute temperature, a straight line is obtained which is of great significance. This has been expressed in the following way by Phipps:

$$\frac{d \ln k}{dT} = \frac{q}{RT^2}$$

$$\ln k = - \frac{q}{RT} + c.$$

where k is the specific conductance of the crystal

T is the absolute temperature

R is the gas constant

c is a constant

and q is the heat of liberation of a gram ion in the crystal lattice, that is, the work necessary to produce a mole of ions in the interior of a crystal.

It is peculiar to these systems that frequently only one kind of the ions is liberated and they carry all of the current. In a series of simple salts with a common anion, the chlorides of K, Ag, Na, etc., in which the positive ion is the carrier, the slopes of the $\ln k$ vs. $1/T$ curves will all be equal. In other words the energy necessary to liberate a positive ion from a chloride lattice is always the same.

If, on the other hand, one deals with a series of simple salts with a common cation, NaF, NaCl, NaBr, and NaI, it is found that the slope of the curve becomes progressively less in the order given, indicating that the energy necessary to liberate Na⁺ ions becomes less the greater the atomic weight of the anion. The work of liberation of an ion is also related to other properties. The following table, adapted from the article of Phipps, Lansing and Cooke, shows it to be closely related to the natural quantum of the crystal as derived from specific heat data.

TABLE I

Relation between Heat of Liberation of a Gram Ion and the
Natural Quantum of the Lattice

Crystal	q (cals)	$q/h\nu$
NaF	32,800	38.2
NaCl	20,200	36.4
NaBr	18,400	42.4
NaI	13,800	38.5

The following sentence is quoted from the article of Phipps, Lansing and Cooke. "As the heat of liberation decreases the quantum of energy decreases correspondingly, so that the number of quanta necessary to activate the Na^+ ion is practically constant for such a series."

The observed conductance effect is not a dielectric displacement (except possibly at very low temperatures), because in that event the effect would change but little with temperature. The current actually transfers charges through distances incomparably greater than atomic and molecular distances. Another important fact to be considered is that many experiments of Joffé and of Tubandt¹³ have shown Faraday's Law of electrolysis to be quantitatively obeyed.

It can be predicted that with a systematic study the number of quanta required to activate the several ions will in each case be constant, that periodic regularities will appear, and further that the laws based upon an ionic conduction in a homogeneous medium will be obeyed. In other words, the electrical behavior of a crystal appears to be quite different from that of the type of substance which has been described as a moisture absorbing dielectric, although many believe the mechanisms in the two cases to be quite similar.

In order to definitely decide such questions as have been discussed, it seems necessary to continue the careful studies of the compositions, sizes and shapes, and arrangements of the aggregated molecules which form the highly polymerized organic substances. It is of more than passing interest that as these scientific problems are being solved, means are indicated by which the electrical characteristics of our ordinary dielectric materials may be improved.

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June 1, 1931.*

¹³ Z. anorg. Chem., **115**, 105 (1921), et. al.

INDUCED OXIDATION OF GLUCOSE IN PRESENCE OF INSULIN ACTING AS AN INDUCTOR

BY HIRA LAL DUBE AND N. R. DHAR

In previous papers¹ we have advanced the view that insulin acts as a promoter in the oxidation of glucose in the animal body. The experimental results recorded in this paper shows that *in vitro* insulin acts as an inductor in the oxidation of glucose in presence of phosphates by passing air, although Spoehr and Smith² did not observe any increased oxidation of glucose in presence of insulin. The experimental details are not available in the paper of Spoehr and Smith.

All our experiments were carried out in diffused daylight inside the room at a temperature of about 25°. In these experiments a slow current of air was passed through a series of bottles, containing 20% sodium hydroxide solution, baryta and concentrated sulphuric acid to free the air from carbon dioxide gas and moisture. This carbon dioxide free air was passed through the solution of glucose containing insulin and other substances, such as ferrous hydroxide, sodium phosphate, etc. A measured volume of air was passed. The insulin used was obtained from British Drug House, containing 5 cc. in the tube and 1 cc. contained 20 units. In every experiment a fresh solution of the insulin was taken, as it was observed during the experiments that the solutions putrifies on keeping. It is practically neutral and does not reduce Fehling's solution. 5 cc. of the insulin (20 units) in the tube was made up to 50 cc. by adding distilled water and this aqueous insulin was used in the following experiments. Extra pure glucose of Merck was used for the experiments. The volume of the solution to be oxidized was always made up to 100 cc. by adding distilled water. 36.5 litres of air were passed in 15 hours.

Experiments with Insulin and Sodium Phosphate

In each of these experiments 10 cc. of 1% glucose solution was taken and it was estimated by the reduction of Fehling's solution and finally weighing the precipitate as cupric oxide.

$$10 \text{ cc. glucose} = 0.2310 \text{ gram CuO.}$$

In the following experiment 0.348 N solution of disodium hydrogen phosphate was used:

No. of experiment	Litres of air passed	Insulin in cc.	Sodium Phosphate in cc.	Actual weight of glucose in 10 cc. of the solution taken in gm. (Blank)	Amount of glucose left after experiment in gm.	Amount of glucose oxidised in gm.	Percentage amount of glucose oxidised
1	36.5	10	10	0.1000	0.0982	0.0018	1.8

¹ Dhar: *Chemie der Zelle und Gewebe*, 12, 217 (1925); *J. Phys. Chem.*, 29, 376 (1925).

² *J. Am. Chem. Soc.*, 48, 236 (1926).

In the following experiments 0.415 N solution of sodium phosphate was used:

2	36.5	10	10	0.1000	0.0987	0.0013	1.3
3	36.5	20	10	0.1000	0.0916	0.0084	8.4
4	73	25	10	0.1000	0.0761	0.0239	23.9
5	36.5	25	30	0.1000	0.0678	0.0322	32.2

In the following experiments no insulin was used:

No. of experiment	Litres of air passed	Sodium phosphate (0.348N) in cc.	Actual weight of glucose in 10 cc. of the soln. taken in gm. (Blank)	Amount of glucose left after experiment in gm.	Amount of glucose oxidised in gm.	Percentage amount of glucose oxidised
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1	36.5	10	0.1000	0.0997	0.0003	0.3
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In the following experiments 0.415 N disodium hydrogen phosphate was used:

2	73	10	0.1000	0.0844	0.0156	15.6
3	36.5	30	0.1000	0.0961	0.0039	3.9

The above tables clearly show that there is more oxidation of glucose in presence of insulin and phosphate than in phosphate alone. This leaves no doubt that insulin acts as an inductor in the oxidation of glucose in presence of sodium phosphate. It is well known that the part which phosphate plays in the animal metabolism is unique.

We have carried on experiments with freshly precipitated cerous and ferrous hydroxides also and the results obtained conclusively prove that they also help insulin in the oxidation of glucose.

The same amount of cerous hydroxide was used in each of these experiments:

No. of experiment	Litres of air passed	Insulin in cc.	Actual weight of glucose in 10 cc. of the solution taken in gm. (Blank)	Amount of glucose left after experiment in gm.	Amount of glucose oxidised in gm.	Percentage amount of glucose oxidised
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1	36.5	10	0.1000	0.0830	0.0170	17.0
2	36.5	20	0.1000	0.0789	0.0211	21.1
3	36.5	25	0.1000	0.0789	0.0212	21.2
4	73	25	0.1000	0.0675	0.0325	32.5

In the following experiments also the same amount of cerous hydroxide but no insulin was used:

1	36.5	—	0.1000	0.0845	0.0155	15.5
2	73	—	0.1000	0.0701	0.0299	29.9

In the following experiments ferrous hydroxide was used instead of cerous hydroxide:

No. of experiment	Litres of air passed	Insulin in cc.	Actual weight of glucose in 10 cc. of the solution taken in gm. (Blank)	Amount of glucose left after experiment in gm.	Amount of glucose oxidised in gm.	Percentage amount of glucose oxidised
1	73	25	0.1000	0.0806	0.0194	19.4
2	73	No Insulin	0.1000	0.0923	0.0077	7.7

We have also carried on some experiments with glucose and insulin alone and we find that there is very slight oxidation. The following results were obtained:

1	36.5	10	0.1000	0.0992	0.0008	0.8
2	36.5	25	0.1000	0.0990	0.0010	1.0

By these results we are led to believe that there is some oxidation of glucose in presence of insulin alone *in vitro*, but by adding cerous or ferrous hydroxides, which act as surfaces the oxidation is facilitated.

We have observed that insulin by itself is oxidized by passing air at 25° and carbon dioxide is evolved, but when glucose is added the oxidation of insulin is greatly retarded. This led us to believe that insulin acts as an inductor in the oxidation of carbohydrates. In several publications¹ we have shown that the slow oxidation of substances can be retarded by another reducing agent, which is slowly oxidized along with the primary reaction. The oxidation of insulin induces the oxidation of glucose.

We have tried the oxidation of glucose in presence of insulin and sodium bi-carbonate and sodium carbonate, but in the case of sodium bicarbonate we find that the oxidation is practically the same in presence or absence of insulin. In the case of sodium carbonate the results are curious. There is more oxidation in presence of sodium carbonate alone than in presence of sodium carbonate and insulin. We have obtained the following results. In the following experiments 10 cc of 1.005 N sodium bicarbonate solution was used:

No. of experiment	Litres of air passed	Insulin in cc.	Actual weight of glucose in 10 cc. of the solution taken in gm. (Blank)	Amount of glucose left after experiment in gm.	Amount of glucose oxidised in gm.	Percentage amount of glucose oxidised
1	36.5	10	0.1000	0.0983	0.0017	1.7
2	73	25	0.1000	0.0987	0.0013	1.3
3	73	No Insulin	0.1000	0.0982	0.0018	1.8

In the following experiments 30 cc. of the normal sodium carbonate solution was used:

1	36.5	25	0.1000	0.0786	0.0214	21.6
2	36.5	50	0.1000	0.0858	0.0142	14.2
3	36.5	No Insulin	0.1000	0.0703	0.0297	29.2

¹ Dhar: Proc. Akad. Wet. Amsterdam, 29, 1023 (1921); Z. anorg. allgem. Chem., 144, 289 (1925).

It is difficult to explain satisfactorily these results.

Since 1922 much experimental investigation has been carried on with insulin and its influence on glucose metabolism in the animal body and it is generally believed that it helps glucose metabolism. From our experiments it is clear that insulin plays an important part in the carbohydrates metabolism *in vitro* and it acts as an inductor. It is easily oxidised by passing air and when mixed with glucose solution the oxidation of insulin is retarded, while it helps the oxidation of glucose. On addition of sodium phosphate or cerous or ferrous hydroxide the oxidation of insulin is accelerated and there is a large amount of carbon dioxide liberated and at the same time the oxidation of glucose is also increased. Sodium phosphate and cerous and ferrous hydroxides facilitate the oxidation of glucose. In the animal body also phosphate is present which must be helping the oxidation of glucose by the secretion of the pancreas. Moreover, surfaces are also present in the animal system.

The behaviour of insulin resembles that of glutathione. Glutathione is an auto-oxidisable substance. It oxidises itself by the oxygen of the atmosphere and at the same time induces the oxidation of the cell constituents. Harrison¹ has shown that traces of iron cause a marked acceleration in the auto-oxidation of glutathione; and hence the oxidation of tissue components induced by glutathione will also be accelerated by iron. The same behaviour is seen in the case of insulin also and our experiments show that insulin, like glutathione is our auto-oxidisable substance, which induces the oxidation of glucose in the animal system.

It is probable that insulin is a polypeptid, a group of substances known to participate in important ways in the metabolism of the body. When analysed by the method of Folin and Looney, there is found 17.9% tyrosin, 7.1% cystin, 0.8% tryptophan and 8.5% histidin in insulin. Insulin is not digested by pepsin nor by trypsin, but in an alkaline medium, in which trypsin is present, insulin becomes inactivated. It may be reactivated, however, showing that it is not destroyed. It appears from our experimental results that in presence of sodium carbonate, insulin is inactivated and does not increase the oxidation of glucose by air *in vitro*.

It will be interesting to note here that Bertrand and Mâcheboeuf² have found that insulin contains very small amounts of nickel and cobalt salts (the amount is never greater than a fraction of a milligram per kilo of tissue). The amount of sugar metabolised under the action of insulin is increased when nickel or cobalt compound is given simultaneously.

The recent results obtained by Svedberg³ seem to demonstrate that insulin is a well-defined protein belonging to the same class as egg albumin and Bence Jones protein. As pointed out by Dr. H. Jensen of the Johns Hopkins University, Baltimore, this fact makes it very improbable that the synthesis of insulin will ever become possible.

¹ Biochem. J., 18, 1009 (1924).

² Compt. rend., 182, 1305, 1506; 183, 5, 257, 326 (1926).

³ Nature, 127, 438 (1931).

Summary

(1) Glucose is appreciably oxidised by passing air through solutions of glucose in presence of insulin at 25° . Phosphates, cerous and ferrous hydroxides markedly accelerate this induced oxidation of glucose by air in presence of insulin.

(2) Insulin is oxidised by passing air and in presence of glucose, the oxidation of insulin is retarded but the oxidation of insulin induces the oxidation of glucose. This is the probable mechanism of the increased oxidation of glucose in presence of insulin in the animal body.

(3) Sodium carbonate appears to inactivate insulin and in presence of sodium carbonate, there is no increase in the oxidation of glucose due to insulin.

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May 24, 1931.*

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